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THE PERIPHERAL CIRCULATION

A. Apéria*

Three matters are of special importance for an understanding of the physics of circulation: a knowledge of the elastic properties of the blood vessels, an appreciation of certain physical properties of the blood, and the theoretical interpretation of results obtained by measurements and recordings. Research during the past two years has made important advances in each respect.

Elasticity of Vessels and Blood Flow

Since the time of Hales, Euler, Young, the brothers E. H. and W. Weber and Frank, we have been accustomed to dividing the vascular system, up to the veins, into two sections. The arteries proper form the predominantly elastic part, physically comparable to the air-chamber [windkessel] in a fire-engine. The arterioles and capillaries give rise to a peripheral resistance, to which the laws of Poiseuille flow are more or less applicable. Frank has attempted, in a simplified way, to analyze the peripheral circulation mathematically. His fundamental formula, arising from the arterial air-chamber theory, was founded on the bases just mentioned, and it consists of a differential equation of the first order, expressing the pressure P as a function of the time t. The

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*From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden. This highly important review was to have been published in the 1944 *Annual Review of Physiology*, but was received too late for inclusion in that volume. Meanwhile other arrangements had been made by the editors for the 1945 volume, which precluded publication of Professor Apéria's article. Accordingly, Professors H. J. Ralston and Eric Ogden of the Physiology Laboratory of The University of Texas Medical Branch, Galveston, offered to seek American publication of Professor Apéria's review, since they felt that it was a distinct honor that Professor Apéria should have undertaken its preparation in English. *Texas Reports on Biology and Medicine* is proud to publish posthumously this very stimulating survey by our brilliant and lamented Swedish colleague. Our thanks are extended to the editors of the *Annual Review of Physiology* and to Professor G. Liljestrand, of the Karolinska Institutet of Stockholm, for their generous coöperation. A translation of Professor Liljestrand's obituary notice of Professor Apéria follows this review. Editorial remarks are enclosed in brackets.

coefficients in this equation are 1/E and 1/W, where E is the coefficient of the modulus of elasticity, and W is the peripheral resistance. [The author, for brevity's sake, frequently refers to E as the elastic modulus. Actually, it should be designated the coefficient of the elastic modulus, since it is defined as dP/dV, while the modulus is defined as dP·V/dV. E is sometimes described in recent literature as the "efficient" modulus, a term which also appears in the author's paper of 1940, and which may have been a consequence of awkwardness in translation.] As a first approximation, these coefficients are regarded as constants.

In 1926 Frank assumed the existence of a "Grundschwingung" [fundamental oscillation] in the air-chamber. With the help of this it was possible to determine the length of the functional air-chamber. This fundamental oscillation requires for treatment a differential equation of the second order. Such an equation was derived in 1932 by Broemser. In 1940 Broemser, using Fourier analysis, described a method for calculating the period T of Frank's fundamental oscillation in cases where it cannot be determined directly from a pressure curve. With this method, if correct, it is further possible to determine the blood pressure amplitude [pulse pressure] for a peripheral curve if one knows the absolute values for a central curve. Broemser's extension of the air-chamber theory, however, stands in need of verification.

It has been shown that the air-chamber theory often disagrees, to a considerable extent, with experimental data. Further studies along this line were made by Deppe and Wetterer in 1939 and by Apéria in 1940–42. The first named writers used an induction tachograph, with the aid of which they directly recorded central flow curves in different animals. [The induction tachograph is an electromagnetic instrument for recording the flow of blood.] In these experiments it was found that the discrepancy between the calculated and the experimental stroke-volume sometimes amounted to over 200 per cent. Wetterer is, therefore, trying anew to revise the original air-chamber theory, and along a somewhat different line than that of Broemser. He assumes the length of the air-chamber to vary during diastole, and to increase with the

pressure wave. If the pulse wave velocity is designated c, the length of the air-chamber will thus be ct, where t is the time measured from the beginning of systole.

Besides certain physiological and physical objections that may be raised against Wetterer's theory, there are also physico-mathematical errors in his main formula. The calculated stroke-volume is often far too much in error.

Wetterer considers only the systolic flow. At the end of systole his air-chamber reaches the volume $V = QcS$, where Q is the active cross-section and S the duration of systole. On the basis of a cylindrical form for the air-chamber, this leads to an abnormal value for the length cS. In order to avoid this physiological absurdity, Wetterer assumes a corresponding transverse enlargement of the air-chamber as soon as it has reached the middle-sized arteries. The total cross-section of the arterial system indeed increases toward the periphery. It may, however, be remarked that in the case of a variable total cross-section of the air-chamber, completely new formulae must be set up for the value of the arterial elasticity.

The question of the air-chamber's relations during diastole is not considered in the above theory. It is left open as to whether one is to understand the total volume QcS to suddenly become equal to zero at the end of systole, or whether one is to consider the volume as gradually diminishing. In the first case one might use the ordinary theory if one does not consider the total cross-section as varying. Here one would obtain an exponential expression for the fall in pressure during diastole, possibly toward some asymptote *a*. (Recklinghausen, Whittaker and Winton, Gomez.) But will such a treatment of the diastolic pressure suffice for theoretical, or, at least, for clinical purposes?

The answer will depend upon the degree of accuracy one demands. If the fall in pressure toward the asymptotic value *a*, during diastole, is an exponential function, $-dP/dt = g(P - a)$, then the "damping" factor g must be a constant. It has been shown (Apéria, 1940) that this is not generally true in the human subject. Under certain conditions, however, it may be of value to employ an approximately con-

stant g. In any event, the closer investigation of the deviation of the fall in blood pressure from the exponential form is an important phase of hemodynamic research.

Gomez ligated the abdominal aorta in order to produce an artificial prolongation of diastole. The aim of the investigation was to show that the fall in pressure, peripheral to the ligature, proceeded exponentially, gradually approaching an asymptote *a*. Deppe continued Gomez' investigations with a modified method. The ascending aorta was ligated in animals with intact pressor receptors, in animals with the pressor receptors eliminated, and in animals under pharmacological influence. The calculation of g was carried out both with $a = 0$ and an a that was read off empirically from records of the prolonged diastole, taking a at a point where a fresh rise in the curve began. The value of g $(= E/W)$ proved to be insufficiently constant.

In the rabbit, after ligation of the aorta, the lower limit for the value of the pressure amounts to about 15 mm. Hg., while in man, after ligation of the brachial artery, Gomez found a value of 60 to 70 mm. Hg. When the pressor receptors are normal, the limiting value is lower than when the receptors have been eliminated. In the first case one finds, for a given diastolic pressure, a higher aorta-iliac pulse wave velocity than in the second case. The excitation of the pressor receptors leads to an increased value of the modulus of elasticity E in the arterial air-chamber.

During the artificially prolonged diastole E/W diminishes. This is due both to a decrease in E and an increase in W. In order to give these some characteristic value, one should specify a definite time-point. Deppe chooses for this the beginning of diastole, and determines the course of g, using several auxiliary hypotheses, in a series of mammals. The corresponding diagrams are also given. The expression for g as a function of the diastolic period turns out to be a fairly clear hyperbola.

As regards Deppe's view, opinion may be divided as to whether an artificial prolongation of diastole is absolutely necessary for an estimation of the course of the diastolic pressure. The prolonged diastole, in any case, can scarcely be

regarded as physiologically normal. The whole question can be resolved under basal conditions (Apéria).

The Poiseuille type of flow is always implicit in the air-chamber theory. But the laws for this type of flow do not apply for pressures in tubes that are subject to temporal changes. The linear relation between flow and pressure may, however, be proved for the undulatory theory (Apéria, 1940) on the basis of certain simplifications relating to the state of the elastic arterial wall, even when the limits of the tube are arbitrarily set. The much-debated question as to the "correct" limits of the air-chamber may thus be resolved in a simple way. The peripheral end of the air-chamber may be shifted arbitrarily toward the periphery provided that the total cross-section be changed only slightly in space and time. The theory can be developed with the use of suitable constants. One is justified in fixing the distal end at, say, the femoral artery.

Frank's air-chamber theory, supplemented with the distal pressure-limit *a* of Recklinghausen and Gomez, appears as the first approximation of the undulatory theoretical continuity equation. With the help of the latter, as Frank (1899) has stated, one can calculate the central flow i from a single pressure curve, as soon as the necessary constants are known. These constants are the elastic modulus E, the peripheral resistance W, and the asymptotic pressure *a*. Several new graphic analytic methods for the evaluation of these constants have been described (Apéria, 1940). Allowance is there made for the viscosity of the blood in the determination of E.

The usual determination of the mean pulse wave velocity *c* really assumes a purely advancing wave. Besides this source of error, it is difficult, in man, to measure this velocity for the aortic arch. In the determination of the stroke-volume based upon the air-chamber theory, the *g* calculated by graphic-analytic methods therefore requires a correction. In 80 cases where the physically determined stroke-volume was compared with gasometric measurements, it was shown that a suitable correction could be made by changing the selected length of the air-chamber in proportion to the duration of diastole (Apéria, 1941). By simple analytic methods one can

approximate the course of every diastolic blood pressure with an exponential curve, without resorting to artificial prolongation of diastole.

Deppe considers such approximations to be inadequate. The goodness of an approximation is a relative concept. The calculation of the best possible approximate constant g is a matter that comes within the sphere of the air-chamber theory. One asks himself, however, whether it is possible in this way to obtain results of practical value. This *does* seem to be the case if the theory is carefully utilized. A physical stroke-volume formula is given (Apéria, 1941), using an approximate value of g, although in an earlier paper the essential inconstancy of g was emphasized. Deppe's polemic (1941) against the calculation of an approximate constant g contains mathematical and physical errors.

The limit of error in the above-mentioned stroke-volume formula was shown to be, in 80 cases, 35 per cent. The absolute mean deviation was somewhat over 11 per cent. Earlier formulae, based on the assumption of a single air-chamber, showed with the same material considerably larger deviations. Wezler and Böger's formula showed an absolute mean deviation of 38 per cent, Broemser and Ranke's formula 65 per cent, and Broemser and Wetterer's formula 93 per cent. The best agreement with these formulae was obtained with young normal subjects. Big deviations were found in higher age groups, in hypertensive subjects, and in individuals with tachycardia.

The formula cited has a usable validity only under basal conditions. In non-basal states, one must consider the possibility of changed reflection conditions of the pulse wave strongly influencing the results. The usual method for the determination of the pulse wave velocity may then be much too great in error.

The question of the limit of error allowable is of great importance in any application of the air-chamber theory. A survey of this question was made in the paper containing the stroke-volume formula (Apéria, 1942). In deriving that formula every attempt was made to avoid using hypotheses auxiliary to the air-chamber theory proper. Such hypotheses

are found to a considerable extent in the stroke-volume formulae given by Broemser, Wetterer, and Wezler. As a rule, they lack sufficient physiological and physical support, and perhaps for this reason they sometimes, even under basal conditions, lead to clinically unacceptable results.

For non-basal conditions, recourse is often made in practice to Liljestrand-Zander's formula, which may be written $V_s = C \cdot \triangle P/P_m$, where V_s is the stroke-volume, C is a constant, $\triangle P$ the pulse pressure, and P_m the arithmetic mean pressure. The individual "constant" C needs to be calculated only once, preferably under basal conditions, for which physical methods are available.

It is a comparatively much debated question as to whether the factor C may really be regarded as an individual constant. It is possible to interpret C as the quantity of blood that through the work of the heart receives, either directly or indirectly, a pressure increment corresponding to the pulse pressure. The question of the approximate constancy of C seems to lie chiefly within the empirico-statistical sphere. A negative stand on this question is taken by Wezler and Thauer (1941). They arrived at this conclusion on the basis of experiments done on nine dogs. As a control check they used the Van Slyke analysis and Fick's principle. In judging the results of their experiments, however, they made a mistake in that they considered C not only an individual but also a species constant. According to their view, C should therefore be the same for all nine dogs, if the Liljestrand-Zander formula is correct. Their results are consequently not valid as a refutation.

Blasius in a statistical study has been able to make a more positive contribution to this problem. He uses the experimental animal data given by Deppe and Wetterer and shows that C can scarcely be considered a real individual constant. He finds, however, a notably good agreement when the Liljestrand-Zander factor $C = V_s\ P_m/\triangle P$ is taken as a linear function of P_m and $p\ (P_m + q)$, where p and q are two new individual constants. p is taken proportional to the body length, and q as dependent on the cross-section of the aorta and the

arterial elasticity. No theoretical explanation, or more precise account of these assumptions is given.

Wezler and Thauer have in their experiments investigated the practical applicability of the stroke-volume formulae of Broemser and Ranke, Wezler and Böger, Broemser and Wetterer and Recklinghausen. The agreement was fairly satisfactory. One may say that the changes in the pulse pressure in their cases do not show such big variations as one often finds in man, especially in cases of hypertension. The limit of error, even so, is occasionally relatively great, in one case 77, 44, 184, and 56 per cent, and in another —25, —31, 39, and —37 per cent, respectively. In the opinion of the above authors the mean error is, however, acceptable. Apéria has shown, however, that in hypertensive human subjects the corresponding limit of error is 100 per cent or higher. One is therefore justified in requiring that the air-chamber theory in its hithereto accepted form be appropriately modified, since errors of the magnitude mentioned are scarcely allowable.

The direction which this modification of the air-chamber theory should take is a question. One should be cautious in employing auxiliary physiologico-physical hypotheses like those of Broemser and Ranke and Wezler and Böger. An air-chamber subject to temporal changes, introduced by Wetterer, does not help, at least so long as several assumptions in the development of the formula are open to question.

Within the sphere of validity of the air-chamber theory, it is possible to determine not only g, a, and V_s, but also W and E. The last magnitude, especially, which is otherwise not susceptible to direct measurement, can be determined purely according to the air-chamber theory as soon as V_s is known. The measurement of the stroke-volume can be carried out gasometrically or, for example, with the ballistocardiograph. Bradley and Parker, in their study of the effect of angiotonin on the circulation, have made use of the formulae in question for the determination of E. These writers correctly emphasize the frequently uncertain approximative significance of all air-chamber calculations. They find the air-chamber theory satisfactory to the extent that E, so calculated, increases

with age, which ought to be required from physiological considerations.

Starr and Gamble make use of an apparatus called the ballistocardiograph, which measures the oscillations of the center of gravity of the body due to the heart beat. An empirical formula for the stroke-volume has been set up on the basis of comprehensive experimental data. The formula, thus far, has been verified chiefly by statistical methods. A theory of the ballistocardiograph is being worked out.

It might, perhaps, be possible to improve the air-chamber if instead of simply a central air-chamber, one were to introduce an air-chamber situated more peripherally, with elastic properties different from those of the central one. Certain investigations (Hauck; Greven and Federschmidt) seem to indicate that such a division is justified. Dillon and Hertzman also consider this question. They use a photoelectric plethysmograph to register the volume pulse of the radial and digital arteries. The method is based on the principle that the opacity of a tissue varies with change in blood content. The volume changes are, where E is constant, proportional to the pressure changes.

In comparing curves from the radial and digital arteries, they find, in contradistinction to the case of centrally located arteries, that the peripheral curve has a longer crest-period or ascent. In certain pathological states, changes in the pulse contour are demonstrable on the digital artery earlier than on the radial. In the interpretation of the prolonged crest-period, certain important factors are adduced: increased peripheral resistance, higher diastolic pressure in the arcus volaris than in the radial artery, and lower pulse wave velocity in the region of the radial and digital arteries than in the nearest proximally located region.

It is of interest to exploit, for special clinical purposes, the results of hemodynamic research. Hildebrand and Möller studied the effects of pressure on the carotid artery in two normal subjects aged 64–65 years. They used the formulae of Wezler and Böger for the calculation of E, V_s, W, and g. In one experiment the cardiac output sank 55 per cent. Such calculated data are perhaps questionable, owing to the uncer-

tainty of the formulae employed. In general, one is probably justified in saying that the results of a limited series of experiments cannot be given too high a validity in circulation questions. The many possible sources of error in such a limited series of cases are altogether too serious to be ignored. Be that as it may, the above authors hold the following view regarding the results they have obtained: "With carotid pressure tests on older, normal persons, one regularly obtains bradycardia, diminution of cardiac output, and diminution of the elastic resistance E. The peripheral resistance showed on one occasion diminution, and on another occasion—with diminished minute volume—an increase."

Wezler and Böger (1939) have suggested a classification of hypertensive subjects on the basis of calculated values of the elastic resistance E [this is the same E discussed earlier], the peripheral resistance W and the stroke-volume V_s. The stroke-volume determined by the formulae of these authors may, in hypertensive subjects, exceed by 100 per cent the values that are obtained gasometrically. On the basis of such calculations, it is clear that one is not justified in speaking of any new kind of "stroke-volume hypertension."

Admitting the uncertainty of hemodynamic formulae, it would nevertheless be desirable to use statistically significant methods as an aid in diagnosis. An attempt in this direction is being made by Wachsmuth who grants, however, that his material is as yet too limited. He employs, moreover, the formulae of Wezler and Böger, which are precisely those yielding, in cases of hypertension, results not agreeing with reality.

A similar treatment of renal hypertension in experimental animals has been undertaken by Bierhaus and Linder. These writers use the formulae of Broemser and Ranke. These formulae are also generally unreliable when the pulse pressure is large (the error in man may amount to 150 per cent). In the paper in question, however, such amplitudes rarely occur. After a single or double ligation of the renal artery the diastolic pressure rose almost as much as the systolic. The formulae employed therefore probably yield fairly acceptable values for the stroke-volume. The determination of the

peripheral resistance W is carried out according to the principles always recognized in the air-chamber theory. In the case of single ligation of the renal artery W rises only slightly; in the case of double ligation, W rises considerably. The authors assume this to be a sufficient proof that the kidney with damaged vessels gives off a vasoconstrictor substance into the circulation.

Precisely on account of the continually increasing clinical and practical applications of hemodynamic methods, it is necessary that the present inadequate foundations of the theory be extended. It would appear that the only correct procedure would be to systematically strive for higher degrees of approximation. The strictly fundamental equations of hydrodynamics, including the undulatory theory, must serve as the guiding line, the theoretical work always being sustained by experimental investigation.

Frank's original theory may be considered a first approximation to the fundamental hydrodynamic formulae. Frank's main equation, as has been mentioned, is a simplified modification of the hydrodynamic continuity equation. The equation of motion, which plays such an important part in undulatory theory is, in the air-chamber theory, represented only by Poiseuille's law. One can, however, attempt to employ, to a greater extent, the fundamental equations of hydrodynamics. Euler, the father of classical hydronamics, in 1775 laid the groundwork for theoretical hemodynamics. In Euler's equations the mass-forces X in the circulation were, without further explication, assumed to be zero. [Hydraulic engineers speak of the forces acting on fluid masses in fluid flow as those of pressure, inertia, gravity, elasticity, viscosity, and surface tension. In the development of the fundamental equations of hydromechanics—the Euler equations of acceleration—, the last three named forces are arbitrarily set equal to zero. It is to these forces that the author is referring.] Such forces may in part be of molecular origin, and in part be called forth by an external field of force. The latter seems, according to Müntz, to be inherent in the changes of tension of the elastic arterial walls. These certainly, by extension and

contraction, exercise an indirect influence on the movement of the particles of blood. By introducing an elastic potential F, Euler's hemodynamic equations are extended. For the axial mass-force in the vessel, one has $X = -dF/dx$, where x is a distance along the vessel. A special energy equation is given for F.

The strictly fundamental equations are, however, much too involved to be applied in any pratical way. In collaboration with Müntz, Apéria therefore proposed a simplified treatment of the elastic mass-force X. According to the hypothesis advanced, the accelerating effect of the wall tension on the movement of the blood is taken as proportional to the negative "storage-intensity," that is to say, equated to $-h\,dP/dt$, where h, the "extension factor," is a sort of measure of the longitudinal influence of forces from the walls on the movement of the blood. The viscosity is expressed, for the purposes of simplification, as $-u\,dP/dx$ [dP/dx is the pressure gradient], the proportionality constant u being approximately determinable by experiment (Müller). The quantity $1 - u - hc$ (c is the pulse wave velocity) can be calculated from direct hemodynamic measurements, and h thereby determined. This latter quantity may be considered an index of the longitudinal elasticity of the blood vessel. Practical applications in this direction have not as yet been made.

The undulatory theory should be regarded as the real "royal road" in hemodynamics. A paper by Hamilton and Dow shows that this "road" is susceptible to experimental test. For such a test one must, in contradistinction to the simple curves in the air-chamber theory, employ whole series of curves. This has been achieved by simultaneously introducing cannulae into the aorta by way of the carotid and femoral arteries. The theoretical question that is to be at least approximately answered, in this connection, is whether or not the movement of the blood has the character of a d'Alembert wave; i.e., a superposition of a back-and-forth moving wave with the same pulse wave velocity. Hamilton and Dow proceed from the tacit assumption that $X = 0$, which is the case in the usual homogeneous undulatory theory according to

Weber and v. Kries. In their system of curves they show in an interesting (if not quite strict) way that the wave has the above-mentioned character to a notable degree. Standing waves with assignable nodal points can arise, as shown in experiments on dogs.

Flow through Tubes

The hydrodynamic factors in the circulation appear clearly in matters relating to the physical nature of the blood. Problems touching on the blood's viscosity, heterogeneity, etc., are almost unapproachable by purely theoretical methods. A comprehensive experimental approach is, however, possible.

"Blut ist ein ganz besonderer Saft." One is reminded of this dictum from Faust when one studies Müller and Suter. In these papers the blood is treated as a viscous mixture, especially as regards its flow through tubes. For this purpose Poiseuille's law is used, the apparent viscosity coefficient u′ being calculated from the pressure gradient and the mean velocity in a given tube. For arbitrary mixtures, u′ generally turns out to be different from the corresponding coefficient u of the prevailing fluid. u′ is generally greater than u for suspensions and emulsions. The relative increment $d = (u' - u)/u$ provides a sort of quantitative criterion of the degree of influence that has been exercised on the main fluid by the added material. In general one finds that u′ (and therefore d) is not dependent merely on the nature of the mixture, but also on the width and length of the tube and on the mean velocity. The concentration of the added material and the form of the suspended particles exert a strong influence. The investigation is further complicated by the fact that in the beginning of the tube there is an "Anlaufstrecke," or "start-portion," whose length may amount to about 40 times the diameter of the tube, where the velocity profile is not yet stabilized.

The treatment of such a mixture by Poiseuille's law leads, even with laminar flow, to a certian inconsistency, as all such mixtures ought not to be regarded as real fluids. A suspension of flat rubber cylinders in glycerine of the same specific weight may not, strictly speaking, be treated with the usual

hydrodynamic equations. An exact treatment is, at present, impossible. The use of the purely hydrodynamic Poiseuille formula is, on principle, not allowable, and if one takes this law as the basis of his investigations, the results can only be considered valid as regards their general direction. But where the subject is of such inherent difficulty, even this much is of interest.

Müller also investigates turbulent flow. Such flow occurs in the circulation of the blood. The conditions associated with this type of flow are even more complicated than those in laminar flow. The above writer advances a theory of turbulence of his own, as opposed to the classical theory of Reynolds. According to Reynolds, turbulence arises at higher velocities due to the increase of small initial disturbances, resulting in a loss of the stability which obtained at lower velocities. According to Müller's theory, every fluid is held together, by molecular forces, in a local and easily destroyed structure. Changes in the forces in question that occur in the "start-portion" affect, by traction, the streamlines of the fluid. This effect will be the greater, the greater the mean velocity of the fluid particles. If the traction exceeds certain limits, the friction that exercises an inhibiting influence can no longer compensate for the traction. The fluid threads are torn, and radial components arise. The common empirical formulae for turbulent flow are, without further ado, used in the new theory.

Müller's comprehensive experiments include continual changes in the fundamental conditions. Variations are introduced, for example, in the length and cross-section of the tube, the size and concentration of the suspended particles, and the mean velocity of the fluid. The local pressure gradient is read off, and u′ and d calculated.

It is probably still too early to draw any general conclusions from the experiments mentioned. From the physiological standpoint, it is of interest that d turns out to be much less for blood than for a suspension of rubber in glycerine. The d of blood is rather comparable to that of an emulsion of oil in water. The blood thus shows a physical resemblance to a suspension of easily deformable bodies.

Some data may be presented to indicate another direction in which Müller's quantitative investigations (1942) extend. The movement of the blood through a definite circular-cylindrical tube of a given length (cm.) is accompanied, for every velocity U (cm/sec), by a certain pressure gradient dP/dx (cm. Hg./cm.) due to friction. For a tube having a cross-section corresponding to that of the aorta, one finds for U = 50, 100, 150, dP/dx = 0.00368, 0.0841, 0.1681, respectively. For cross-sections corresponding to medium-sized arteries, and U = 5, 20, 50, dP/dx = 0.112, 0.510, 0.964, respectively. For small arteries (diameter 100 micra) and U = 3, dP/dx = 39. For arterioles (diameter 30 micra) and U = 0.5, dP/dx = 100. The above experiments show how the loss of energy increases with the narrowing of the tube. The relative loss u (see above) is of interest in hemodynamics, although the figures given can be considered valid only as regards their general direction, since the experiments were carried out under stationary conditions [that is, uniform flow], while the circulation of the blood involves elastic tubes subject to temporal changes.

Among the experiments with suspensions mention may be made of the apparent viscosity coefficient u′ at different concentrations e of the cylindrical bodies introduced. For spherical bodies Einstein found a linear relation between u′ and e. This relation is not valid for blood where the relation seems, according to Müller, to be exponential. In experiments on the flow of blood in capillary tubes Suter also found an exponential relation.

It is possible to modify the above experiments in other ways. In investigations carried out by Goodeve and Whitfield, even the fluid itself has been made to undergo continuous changes during the course of the experiment, in the form of conversion and precipitation reactions. It is possible to describe certain regularities for u′.

The Interpretation of Some Measurements

The difficult nature of the problems encountered in experimental hemodynamics results in the apparatus used by various investigators becoming increasingly more exact and also

more complicated. Almost every one of the previously mentioned writers uses a new apparatus of his own. For the description of these, the reader is referred to the original papers.

Mention may be made of a comprehensive paper by Rappaport and Sprague dealing with apparatus and technical questions. A description is there given of a combination electrocardiograph, stethograph, and sphygmograph. The experiments refer for the most part to purely physiological acoustic problems. Further application of the apparatus to circulation problems seems desirable.

Lian uses a similar apparatus. With its help he simultaneously registers an electrocardiogram, a sound, and a pressure curve. In this way he believes it to be possible to determine the exact value of the diastolic pressure P_d, as explained below. The usual auscultatory method is stated to give too high values for P_d. The method is also said to be usable when it is difficult to determine the diastolic pressure by other means.

Particularly in cases of aortic disease, one hears, peripheral to the pressure cuff, weak sounds at every heart beat. These sounds are audible even when the cuff is under supramaximal pressure. They derive from the pulse above the cuff and are transmitted through the tissues of the arm. They show a delay as compared with the beginning of the pulse above the cuff. If the external pressure is reduced from the systolic P_s to the diastolic P_d, the delay also diminishes and gradually disappears at P_d and lower pressures.

The ordinary systolic sounds, that are transmitted by the blood when the external pressure is lower than P_s, show a similar delay as compared with the beginning of the pulse above the cuff. When the external pressure is higher than P_d, the delay is even greater than in the first-mentioned case. With decreasing external pressure the difference diminishes, and completely disappears at P_d and lower pressures. In the last mentioned pressure range there is no delay even for the sounds that are transmitted by the blood. Above the systolic pressure one thus hears a single sound, transmitted through the tissues; between the systolic and diastolic pressures one

hears double sounds, one sound transmitted by the tissues, followed by the other which is transmitted by the blood; at the diastolic pressure and below, the sounds coincide with each other as well as with the beginning of the pulse.

For sounds transmitted by the blood, an adequate physical explanation is given, though no attempt is made to give one for the other sounds. These observations are stated to be readily demonstrable clinically. It is, perhaps, not yet quite certain whether Lian's investigations can lay claim to general validity. If the alleged regularities prove to be constant, a new method of clinically determining the diastolic pressure becomes possible.

In research on the circulation, it is not so easy to interpret, in a satisfactory way, several simultaneously recorded curves. Even the qualitative interpretation is often not easy. It is of importance, however, to collect material of this sort. Of special interest is the simultaneous registration of several pressure curves from regions close together. Two such curves give, for example, the pressure gradient dP/dx, while three curves yield the change in the pressure gradient with distance; that is, the second derivative of P with respect to x, d^2P/dx^2. This latter value has found some application in the literature.

dalla Torre has constructed a blood pressure apparatus with four isloated air chambers. With this it is possible to record four curves simultaneously. The cuff is 13.6 cm. wide, the separate parts of the cuff being correspondingly narrow. Of the curves obtained, the proximal ones are more sharply pronounced than those obtained with an ordinary cuff. The form of the oscillations appears to make it easier to apply the usual criteria, especially in the determination of the diastolic pressure. The more detailed interpretation of the four curves is at present, however, very difficult.

Even the correct interpretation of a single pressure-cuff curve is a debated matter. An interesting contribution in this direction has been made by Harlé. The theory of the blood pressure cuff is treated according to mechanical principles, but without differential equations. The author does not make use

of experimental material but relies more upon general considerations.

A theoretical diagram is given (figure 1), in which the external pressure P′ is chosen as the abscissa, and the radial displacement y of the arterial wall as ordinate.

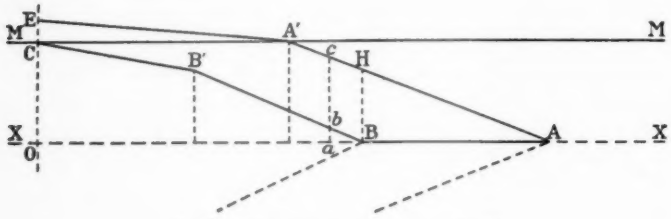

Fig. 1. Diagram according to Harlé

The line XX represents the position of the axis of the artery, and the line MM the normal diastolic displacement of the wall at $P' = 0$. The displacements of the wall at the systolic and diastolic pressures are represented by the curves EA′A and MB′B, respectively. The two curves are represented as each composed of two straight-line portions. The displacement of the wall at the systolic pressure P_s is designated y_s, and the displacement at the diastolic pressure P_d is designated y_d.

As the external pressure P′ diminishes, it finally arrives at a point A where the first systolic "lifting" of the arterial wall occurs. At B the corresponding diastolic point occurs. The systolic curve is represented as showing a break at A′, which is the point where y_s is equal to the normal diastolic displacement.

The artery's resistance to compression is designated r_s for the systolic phase, and r_d for the diastolic phase. Besides these "static" forces, the artery is also affected under the cuff by forces of dynamic origin derived from the cardiac impulse.

For the first phase, represented by the region to the right of point A in the diagram, one can write $P' > r_s + f + f_1$, where f represents a pressure due to the approaching pressure-wave, and f_1 represents a pressure due to the reflected

pressure-wave. At the point A, $P' = r_s + f + f_1$. Since r_s is not known, the external pressure when the blood first forces its way through at A does *not* correspond to the systolic pressure P_s. One is not entitled to draw the conclusion from experiments on post-morten preparations that r_s, *in vivo*, is relatively small. This is probably true only in childhood.

During the second phase, represented in the diagram by the region lying between A and B, a portion of the energy of the blood is expended in opening up the artery under the cuff. This may be represented as $k \cdot P_s$, where k is a number lying between 0 and 1 as limits. One can then write $f_1 = k \cdot P_s + f_2$, where f_2 represents a residual pressure. Hence

$$P' = r_s + f + k \cdot P_s + f_2.$$

During the third phase, represented in the diagram by the region lying between B and 0, the blood flows through the vessel under the cuff during the entire pulse period. For the diastolic curve one writes, by analogy with the preceding equation, $P' = r_d + f + m \cdot P_d + f_2$, where m is a number lying between 0 and 1 as limits. During diastole there is no dynamic source of force. Therefore one writes, for diastole, $f = f_2 = 0$. No allowance is made for the fact that the elasticity of the vessel acts to drive the blood towards the periphery. Actually, therefore, f and f_2 do not disappear during the period mentioned.

The slope of the line A'A, $-dy_s/dP'$, corresponds in a way to the systolic radial elasticity. An analogous role for the diastolic curve is played by $-dy_d/dP'$. The first of the stated coefficients is, from experience, the lesser. The artery is therefore softer at P_d than at P_s, and consequently makes its greatest oscillation at point B. If the difference between the stated coefficients is small, one finds no pronounced maximum for the oscillation of the wall of the artery. In the oscillogram one sees a plateau instead of a peak. The diastolic de-collapse point B lies, in this case, at the beginning of the plateau.

Clinically, higher values of A or B generally mean higher values of r_s and r_d, respectively, or, in other words, diminished elasticity of the vessel.

It is supposed that when the external pressure is equal to the diastolic, one has $r_s = f = f_2 = 0$, and for lower pressures r_s becomes negative. The argument given in this connection is, however, not sufficiently conclusive. Harlé thinks that in this way he can assure the determination of P_d at the point A′.

For actual practice, what is decisive is how without real oscillograms one is going to determine the points A, B, and A′. We have at our disposal only the oscillograms given by the usual apparatus, which we must use despite its imperfections.

Without any real proof, it is assumed that with diminishing external pressure, the point A for the systolic de-collapse is to be found at the spot in the empirical oscillogram where a sudden increase of amplitude occurs. But the compression then exceeds the systolic pressure by the amount r_s. The point B for the diastolic de-collapse is to be found at the greatest amplitude or at the beginning of the plateau if such occurs. The diastolic pressure P_d at the point A′ is found where a sudden diminution of the amplitude occurs. The softer the artery, the more pronounced will be the changes in slope of the amplitude curve. The breaks shown in the diagram are always accompanied by a change in the direction of the resultant force.

In a third paper Harlé intends to complete his investigations. To the many theoretically still unclear applications of the oscillogram we can now add another by Guillaume. This writer makes use of a diagram that is obtained by the elimination of the time factor. The abscissa represents the external pressure P′ and the ordinate the corresponding oscillation Z. In this diagram there is for a minimal pressure P_1 a corresponding minimum $Z = 0$, and for a pressure P_2 a maximum Z_2. The quantity $(P_2 - P_1)/Z_2$ is designated by the author "l'index tensio-systolique." This index is supposed to characterize the degree of pathological affection of the blood vessels. On the basis of over 200 cases the author believes he is able to state the diagnostic significance of the index.

The Circulating Blood-Volume

An extension of our knowledge of the blood-volume is of importance. A survey of this question has been made by Bazett. He bases his arguments partly upon his own experiments.

One may proceed, among other ways, from the axiom that the blood-volume and the capacity of the blood vessels must, on a given occasion, be the same. In addition to the reflex transference of blood that occurs between different vascular regions, the total circulating blood-volume normally changes in different physiological states. Variations of the blood-volume such as occur with acute changes in the external temperature, after meals, with changes of position, etc., do not usually exceed 10 to 15 per cent. In dehydration due to a hot bath we may find a reduction of 18 per cent. In congestive heart disease, on the other hand, the increase may average 40 per cent.

Primary changes in the blood-volume to which the vascular system adapts itself consist firstly of a reduction, as in hemorrhage and surgical shock, and secondly of an increase, as in blood transfusion. Primary changes in the vascular system, leading to an increase in blood-volume, occur in heart disease and hyperthyroidism. Diminution of the vascular system is found in myxedema. Great importance is attached to the large veins in their function as reservoirs, and also to the arterioles.

The relation between blood-volume and different hemodynamic quantities is mentioned. For example, a dehydration of 3.1 per cent due to a hot bath led to symptoms similar to those occurring with hemorrhage. The reduction in the effective blood-volume was due partly to dilatation of the blood vessels of the skin. The pulse rate increased, the stroke-volume decreased, the cardiac output was subnormal, the blood pressure increased, as did also the peripheral resistance. The aortic elasticity, on the other hand, calculated from the pulse wave velocity, was diminished.

Starling's hypothesis concerning the balance of fluids proves to be of importance for the value of the blood-volume. The

volume of the blood plasma is determined by the total amount of plasma protein circulating in the blood vessels. This amount determines the fluid volume found at a given level of the blood pressure. There seem to be seasonal variations in the concentration of plasma protein, and these variations are related to the incidence of circulatory insufficiency in hypertensive subjects.

Veins, Capillaries, and Arterioles

Szekely has measured the venous pressure during and after exercise. The measurement of the rise in venous pressure during exercise and the period required for its return to the normal value is of a certain practical importance in the estimation of the functional capacity of the circulatory system. In normal subjects the venous pressure returns to its initial level within 30 seconds after exercise. In case of heart disease, the rise is more pronounced, and continues for a longer time. In certain cases of latent congestive heart disease an opposite reaction was noted—the venous pressure was subnormal after exercise.

Linton et al. in experiments on animals, have shown that the ligation of a large vein in one of the extremities increases the arterial flow to the extremity. The increase bore a certain relation to the venous pressure level.

In cases of venous occlusion one finds retrograde enlargement of veins, venules, capillaries, and arterioles. Through increase in the diameter of the vessels, resistance is lowered. Another way in which blood flow may possibly increase is through the opening of arterio-venous shunts, due to the changed distribution of intra-vascular pressures.

Glasser and Lesser have ligated the femoral vein in a number of patients with peripheral vascular disturbances. In the majority of cases signs of improved blood flow were observed. The effect is assumed to be due chiefly to the opening of previously inactive collateral vessels. In a couple of cases this was demonstrated by X-ray.

Wiggers reviews the probable mechanisms underlying peripheral vascular disturbances. The capillaries are referred

to as, in certain respects, the "keystone" of the circulation. The blood flow in the capillaries is regulated by the pressure in the small conducting arteries, the size of muscular arterioles, possibly active changes in the size of the capillaries themselves, the pressure in the surrounding tissues, the pressure in the small venules, and the blood viscosity. Circulatory failure, in the broadest sense, develops when the driving pressure in the small arteries is not sufficient to overcome the composite resistance in the capillary area. In this case, the blood flow diminishes. As an example, the situation in congestive heart disease is mentioned, where the arterial pressure is at a normal value but where the resistance is high due to an elevated venous pressure.

Shock is treated as a special form of peripheral vascular insufficiency, characterized by a progressive fall in both the arterial and the central venous pressure. The venous return is reduced and capillary stagnation occurs. As a consequence, the stroke-volume is reduced. As causes of the reduced venous return, reduction in the volume of circulating blood and retention of blood in the capillaries and sinusoids are adduced. Such a reduction in blood-volume occurs, for example, with hemorrhage or dehydration. Four possibilities have been considered in the literature to explain the capillary stasis: primary arteriolar dilatation, primary arteriolar constriction, primary atony and dilatation of capillaries, and primary failure of some veno-pressor mechanism.

Several of the assumptions mentioned seem not to have been sufficiently clearly analyzed. For example, the vasoconstriction that is visible in the superficial vessels need not necessarily be a contributing factor in shock, but may constitute one of the mechanisms whereby normal circulation is reëstablished. Not enough attention has been paid to the question as to whether a disturbance in the numerous stabilizing mechanisms normally available to the organism constitutes an essential factor in the development of shock.

Werle and Costy have shown that if the blood pressure in a dog is kept for two hours at a level of 65 mm. Hg., by bleeding or plasmapheresis, the reinjection of all the fluid

either completely restores the animal to health, or else results in a circulatory collapse after a temporary improvement. Pressure curves from the aorta in the dogs that were not restored perhaps indicate a disturbance either in the mechanism that changes the size and elasticity of the aorta with varying blood pressure and volume, or else an insufficient adaptation of the myocardium to diminished coronary flow.

As another example of the circulatory adaptability of the organism, Wiggers mentions the adaptation that takes place in connection with arteriolar dilatation. The total venous return, in this case, depends to a large extent upon the ability of the body to mobilize fluid from reservoir organs as a compensation for the "hemorrhage" into small vessels. In this way a normal or increased venous return may follow the administration of nitrite. Histamine, on the other hand, reduces the venous return.

In connection with reduced venous return, certain important extra-vascular factors are adduced, such as support offered by the tissue elasticity, the pumping action of respiration, and active venomotor changes. It is of importance that experimental hemodynamics should indicate the possibility of the appearance of external forces that affect the circulation. In X-ray studies carried out by Bullo it was not possible to demonstrate any active movements of the walls of peripheral veins. It is obvious, however, that such active external forces are exerted upon vessel walls in connection with many physiological functions, such as in digestion, in respiration, and in muscular exercise.

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ARON APÉRIA (1904–1943)

G. LILJESTRAND*

On June 22, 1943, two days after his thirty-ninth birthday, Aron Apéria, a member of this society since 1940, died after an illness of more than a year during which he suffered with heroic patience. In the spring of 1942 a cancer of the colon was discovered in connection with a peritonitis. The malignancy was later operated upon and treated by X-ray therapy. There was a temporary improvement and Apéria eagerly retured to his research, but his time was tragically short.

Apéria was born in Helsingfors but moved to Stockholm when very young. Here he obtained his elementary school training. After this, he was engaged in practical agriculture for a few years. During this period he became interested in poultry breeding and crop rotation among other things. A keen desire to improve his knowledge caused him to work toward a senior high-school examination which he passed in 1927 after several years of study. He then entered medical school and became a "Candidate of Medicine," in 1930, a "Licentiate of Medicine" in 1934. He obtained both degrees in Stockholm and presented his thesis for a degree of Doctor of Medicine there in 1940. His thesis was entitled *Hemodynamical Studies*. His promotion to Doctor of Medicine took place two years later since he had to take a leave of absence because of his illness. His practical training was obtained at Maria Hospital where he worked as a visiting physician. A considerable part of his scientific work was done there.

Apéria had an outspoken desire for research and his creative spirit was well suited to this type of work. A sharp intellect, a rigorous critical attitude and an unusual ability to work combined with modesty and a disregard for worldly acclaim were his distinguishing characteristics.

His first scientific work was carried out in collaboration with E. Carlens (1931). This work was designed to analyze

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in detail the increase in volume of the blood stream after administration of food. This effect had been demonstrated by several investigators. Apéria and Carlens, using Grollman's acetylene method, determined the cardiac output per minute under resting conditions, under fasting conditions and after ingestion of 50, 100, and 150 grams of sucrose; they also measured it after ingestion of an amount of fat isodynamic with the smallest amount of sugar, in the form of 47 grams of butter; and finally, after ingestion of a nearly isodynamic quantity of egg white (80 grams). They found that sugar caused a rapid increase in the cardiac output per minute, reaching a maximum after about an hour. With an increase in dose the duration of the effect was prolonged, but the absolute intensity remained unchanged. After ingestion of fat, the increase in cardiac output appeared more slowly and was more persistent, showing a maximum after two or three hours; egg white caused an increase of remarkably long duration. The total increase of the cardiac output after ingestion of the isodynamic amounts was: for sugar, 55; for fat, 100; for egg white, 240 liters. This work is remarkable for its high accuracy and it could be accomplished only because of Apéria's unusual persistence, due to the considerable experimental difficulties. These facts are very important and are good proof of the heavy burden which a diet including egg white lays upon the heart as compared with a diet of carbohydrate and fat.

This investigation acquainted Apéria with the physiology of circulation, and it absorbed all of his interest from this time on. He affectionately devoted his time to it for the rest of his life.

The Grollman method was used for an investigation concerning the circulatory mechanism of workers exposed to an atmosphere containing esters of nitric acid. These esters play an important role in the manufacture of explosives. The increase in cardiac output per minute due to these nitric acid esters had been observed earlier in experiments under extreme conditions, and could now be demonstrated under moderate exposure. Since nitric acid esters act as vasodilators, this increase in cardiac output may be considered as compen-

sation by the regulatory mechanism of the body for the fall in blood pressure caused by the vasodilatation. Gradually a certain adjustment in the body occurred but at the same time the increase in cardiac output per minute became more and more persistent. Not a single case of hypertony (hypertension) was encountered in the personnel examined. The fact that the effect could be sustained over a relatively long period of time opened the possibility of using these compounds for therapeutic purposes in hypertension. For this purpose Apéria (1938) designed a chamber of 15 cubic meters capacity in which a solution of 80 per cent nitroglycerol and 20 per cent ethylenelgycol nitrate adsorbed on a fine powder was left for evaporation until a concentration of the gases amounted to 3 to 5 mgm. per cubic meter. Here the patients had to remain for 10 to 15 minutes every day or every second day at first. Later the treatment was extended to thirty minutes or an hour depending upon the fall in blood pressure. Apéria obtained good results in 100 cases treated this way. Many patients who had been subjected to a variety of therapeutic measures showed a more or less stable and sometimes remarkable decrease in blood pressure after treatment in this chamber. Their personal discomfort was relieved at the same time. There is no doubt that this method deserves continued examination.

In a subsequent investigation using Grollman's method the cardiac output per minute was determined under standard conditions on 111 adults of varying ages. The results demonstrated an increase in the arterio-venous oxygen difference; i.e., a more complete utilization of the oxygen of blood in the peripheral vessels, which was directly proportional to the increase in age. The cardiac output per minute and per square meter of body surface, the so-called "cardiac index," decreased with age. The differences were not large but they were statistically demonstrated for the extreme groups, and were probable for those in between. At the same time, an increase of the blood pressure amplitude was noticed. This has to be considered as a result of a decrease in the elasticity of vessels which develops gradually. A comparison of normal persons and alcoholics with patients having adiposity or hypertension was also carried out. In alcoholics, a decrease in the cardiac

index could be demonstrated with certainty while the index remained unchanged in the other two groups.

During these investigations Apéria concentrated his interest on the theory of hemodynamics more and more. It became clear that this difficult field required special mathematical training. In accordance with the proverb "What one fool can do, another can," he decided to acquire the necessary ability. Extensive study for several years familiarized him with the mysteries of differential and integral calculus. Then, too, he was able to collaborate with an expert on higher mathematics, Professor H. Müntz. He devoted his efforts almost fanatically to problems in this field. Most of his results are presented in his thesis. It is impossible for me to do more than briefly stress its valuable contents. The thesis is on such a level that it was necessary to call the professor in the field of water motors and pumps from the Technical Institute as an expert. He had no essential criticism to make.

Some earlier investigations of this field had been made. The ones carried out by O. Frank and his school in Munich were especially interesting. Knowledge of the physical factors in blood circulation is of great interest from several viewpoints. The system of vessels may be considered as consisting of a bellows which corresponds to the more elastic parts of the central arteries and a network of pipes which distribute the blood. In the 'bellows' an accumulation of blood occurs during systole; during diastole this accumulation is emptied into the 'pipes,' which in turn mainly determine the resistance. The accumulation depends on the elasticity of the vessels, which decreases considerably with age. This elasticity cannot be measured directly, but the less it is, the faster the pulse rate. Therefore, information concerning the rate of the pulse wave is important. The fall in blood pressure during diastole is a measure of the ratio between the elasticity of the vessels and the peripheral resistance. The dimensions of the bellows and the diameter of the aorta are important. Upon consideration of these and other factors one is in a position to calculate the amount of blood driven out with every contraction of the heart; i.e., the stroke-volume. Several such formulae have been published. The advantage of this method

over gas analytical techniques is claimed to be that coöperation from the patient is unnecessary and that determinations can be repeated at shorter intervals.

Apéria has critically surveyed the basis of these calculations. He has demonstrated that earlier investigators had used over-simplified methods whereby a considerable uncertainty was introduced. Constant pressure in the 'bellows' had been assumed; Poiseuilles' law of liquids streaming in capillaries was thought to hold true for arteries. None of these assumptions is correct according to Apéria. In his opinion even the foundation on which the mathematical theory had been built is too uncertain. He also believed that the mathematical treatment of the problem as carried out previously was too indefinite and involved larger errors.

Because of this controversy, Apéria's next accomplishment was very interesting. He compared the results given by calculating the cardiac output by a variety of formulae given in the literature with the results obtained using Grollman's gas analytical technique. It turned out that he obtained large discrepancies. In certain cases the calculated value was three times as large as that obtained experimentally. He was justified in pointing out that calculation of the cardiac output from physical measurements is not a satisfactory solution to the problem.

His thorough knowledge of the gas analytical method for determination of the cardiac output makes this comparison very valuable. I am eager to point this out, since the Munich school in a recently published paper claims that the very low values for the cardiac output obtained by Apéria in certain instances indicate poor technique. These gentlemen have mistakenly assumed that the experimental material consisted of healthy individuals only; actually Apéria particularly points out that he has included cases of tachycardia with the others. Obviously these cases were included in order to test the physical methods under extreme conditions. In my opinion this work of Apéria represents a highly qualified and valuable accomplishment.

On the basis of his investigations Apéria has given a new formula for the calculation of cardiac output. Compared with

Grollman's method in 24 cases, it gave an average deviation of only 30 per cent. This comparison was later extended to include 80 cases. The average deviation then was only 11 per cent, and discrepancies amounting to more than 25 per cent occurred in just a few cases. Thus the new formula was proved to be superior to those given earlier.

Apéria was not a master of form. His papers are hard to evaluate. The difficulty is due both to his way of presenting proof and to his somewhat clumsy composition. This handicap was even more noticeable in lectures. The work itself is of permanent value, however, and there is no doubt that Apéria has made an important contribution. His death means the loss of an eager, enthusiastic scientist who had acquired by unusual training the best qualifications needed in the investigation of an important field. The loss is very serious since there are few in this country who are in a position to continue the interrupted investigations. He leaves behind the impression of a fine and noble personality who did his job quietly and well. May he rest in peace.

THE EFFECT OF SPASMOLYTIC DRUGS ON THE ISOLATED URETER*

DONALD SLAUGHTER, T. U. JOHNSON, N. TOBOLOWSKY, AND REX VAN DUZEN

Studies on the *isolated* ureter have been made chiefly by Macht, Gruber, and Roth. These investigations may be briefly summarized as follows:

Macht found in pig and human rings that the rate and tonus of the ureter are increased by epinephrine and that large doses induce tetanus. This action is reversed by previous application of ergotoxin. Pilocarpine and physostigmine stimulate the rate and force of ureteral contractions and increase the tonus of the excised ureter. Atropine in suitable doses antagonizes these actions. These same effects were noted *in situ* in the rabbit. Morphine and its chemical allies increase the contractions and produce a greater tonicity of the ureter. Papaverine and its allies produce a slowing or inhibition of the contractions and the tonus. Nitrates rapidly paralyze ureteral rings without completely relaxing them. There was noted on occasion a preliminary stimulation preceding the paralysis. Macht also used hydrastin and emetin on the pig ureter and showed that they exert a papaverine-like action.

Gruber observed that epinephrine increases the rate of conduction of peristalsis and anti-peristaltic waves in the ureter and to the same degree in both types of peristalsis. His work on longitudinal excised pig ureter in general confirms that of Macht.

Roth noted that ephedrine is mainly a stimulant on the excised pig and dog ureter, while epinephrine is 100–200 times

*This work was made possible by a grant to one of us (D.S.) from the Ciba Company, Summit, New Jersey. A synopsis was read by (V.D.) at the Southern Clinical Medical Meeting held in Louisville, Ky., November 14, 1940. A summary of this work was published in the June, 1942, issue of the Southern Medical Journal. Received for publication November 20, 1944.

as actively stimulant as ephedrine. He found that pilocarpine has a questionable stimulating effect while atropine gives no effects.

In research on the intact human ureter, the work of Jona and Flecker, Herbst, Rattner, Ockerblad, and Carrol should be surveyed.

Jona and Flecker directly observed that atropine decreases the activity and causes relaxation of the renal pelvis to the point of producing painful distention. This is relieved by physostigmine (eserine). They further stated that eserine, morphine, strychnine pituitrin and ergot definitely stimulate the motility of the pyelo-ureteral junction.

Herbst states that only eserine relieves post-cystoscopic urinary tract pain. Probably he felt the relief is due to stimulation of an atonic renal pelvis.

To our knowledge the first mention of the action of morphine on the intact human ureter was made by Trattner. He found that the usual clinical doses of this drug markedly increase the tonicity and frequency of ureteral contractions and that atropine decreases these contractions. Ockerblad confirms these findings and states further that the larger the dose the greater the effect. He also points out that atropine antagonizes this action of morphine on the human ureter, but that when given alone, atropine has little effect. In a later paper, Ockerblad and Carlson substantiate their earlier work and point out that unless larger than ordinary therapeutic doses of morphine are given, the pain due to stone or spasm may actually be increased. In certain instances (hydronephrosis in particular) ordinary doses of morphine may bring about relief from pain by forcing, through stimulation of peristalsis, some urine through the obstruction. When large doses are used the relief of pain is effected solely through the central nervous system.

By means of the hydrophorograph, Carroll and Zingale confirmed the work of Ockerblad et al. on morphine and atropine and pointed out that prostigmine and acetylcholine increase the amplitude and frequency of ureteral contractions. They state that "trasentin" and "theelin" have no beneficial relaxing effect on this type of increased ureteral activity

but that a pancreatic extract marketed by Sharp and Dohme causes a complete cessation of uretral waves within a minute or two after administration.

Physiology of the Ureter

Innervation: Most anatomists are now agreed that the ureter has both sympathetic and parasympathetic fibers throughout its extrinsic nerve supply. Further, Lazarus quotes Wharton as demonstrating an intrinsic nerve supply within the ureter. It is generally believed that the sympathetics are primarily motor to the ureter but the use of proper drugs has shown that the parasympathetics are also motor. We propose to prove more conclusively that such is the case.

Muscular activity: The ureter is known to have an outer longitudinal and inner circular muscle, but these interlace. Further, most workers believe that the middle and bladder thirds of the ureter give better contractions (both excised and intact) than does the kidney third of the ureter.

Clinical Use of Anti-Spasmodics

Macht relates how Geraghty first injected papaverine through a ureteral catheter and noticed that the ureteral meatus dilated. Many tried to make use of the relaxing power of papaverine and thus facilitate the passage of ureteral calculi. The results were at times specatacular but at other times disappointing.

In 1925, one of us (V.D.) called attention to the relief obtained by giving atropine or belladonna in cases of ureteral spasm. No beneficial results in cases of ureteral calculi were noted.

Results from pancreatic extract and "Depropanex" were reported by Lazarus and later by Carroll and Zingale. Intramuscular injection gave relaxation of the ureter at times but the effect was not as constant as most urologists desire.

One of us (V.D.) called attention to the relaxing effect of "theelin" in cases of ureteral spasm occurring just before menstruation or in women who do not menstruate but continue to have cycles of ureteral colic. No beneficial results

have been noted in other types of ureteral pain or spasm. We have known for many years that there is a relaxation of the ureters during pregnancy. Jenkins and Van Wagenen have recently shown that this ureteral relaxation occurs in monkeys in whom the fetuses have been removed. They subsequently showed that the relaxation disappears after removal of the placenta.

One of us (V.D.) has used trasentin both orally and by intramuscular injection and has been well pleased with its relaxing effects in cases of ureteral spasm.

In cases in which prompt relief is desired, intramuscular injection of trasentin is employed. It has been very useful in ureteral colic caused by ureteral calculi unless they are impacted. It has been claimed that the drug should be given intravenously. The incident excitement and loss of orientation precludes the advisability of the intravenous route of administration. Some state it should be administered very slowly, taking at least three minutes for injection but from our experience it is to be doubted if the average practitioner will assume responsibility for intravenous injections. One of us (V.D.) is well pleased with the results from oral administration and by intramuscular injection in selected cases.

Reasons for Doing Work

Because of certain discrepancies in the experimental pharmacology of the ureter and in the clinical application of reported drug effects a systematic study of ureteral "stimulants" was indicated. We are particularly concerned, however, in this paper with the action of ureteral anti-spasmodics and the application of them to clinical use. We feel that in the past there has not been a consistent effort to study the effects of spasmolytic drugs in relation to the action of the most important stimulants in one composite set of experiments. We decided to investigate several "anti-spasmodics"; namely, methylamino-methyl heptene (trade-named "octin"), deproteinated pancreatic extract (trade-named "depropanex"), and the phenylcyclohexyl ester of acetic acid (trade-named "trasentin"). These agents and placental estrogenic hor-

mones have not, so far as we know, been investigated experimentally in regard to their action on the ureter.

Technique

The results of the present investigation of the behavior of ureteral tissues are based upon a series of studies upon surviving excised ureters of lower animals. Segments of the excised ureters were placed in a physiological solution through which a constant stream of oxygen bubbled. The receptacles containing oxygen tubes, solution, and ureteral segments were immersed in a constant temperature bath. The lower end of the segment was attached to the oxygen tube in each receptacle; the upper end being connected by light linen thread to lightweight balanced gravity levers arranged to write on a smoked kymograph drum.

By far the most important single technical consideration involved is the composition of the nutrient solution. Macht, Roth, and Gruber have admirably reviewed this subject. They point out that a Locke, Locke-Ringer or Tyrode type of solution is most satisfactory. Macht added urine or urea to produce a more approximate "physiological" medium and Gruber noted the effect of urea on ureteral contractions. After experimentation, we chose as a prototype a Locke-Ringer solution; i.e., NaCl, 9.2 gm.; $CaCl_2$, 0.24 grm.; KCl, 0.42 gm.; dextrose, 1.0 gm.; and $NaHCO_3$, 0.050 gm. in each 1000 cc. To this we added 3 gm. of urea.

There is considerable controversy concerning the appropriate pH of ureteral bath media. A survey reveals that pH ranges of 7.5–8.1 have been used by other workers. We found by trial that the adjustment of pH to 7.4 gives the most consistent results.

We feel that a standard percentage of urea is superior to the addition of urine which varies in its urea content. Further, 0.3 per cent urea approaches a close physiological ureteral fluid.

The dextrose and bicarbonate are best added after the other salts in solution have been adequately oxygenated. On occasion such a finished solution was used after 24 hours' stor-

age in the ice box. As a rule, however, solutions used on a given day were made on that particular day.

The temperature of the electrical bath was kept at 37.5–40° C. We did not find that temperature variation within the range we employed has any appreciable effect on ureteral motility. Below 35° C., the ureters are not very responsive. This agrees with Roth's observation.

Inasmuch as the objectives of the present investigation were concerned with the primary, unmodified effects of certain drugs, the presence of any substance potentially or actually compromising the action of these drugs would have vitiated the results Hence, the desirability of preliminary anesthesia was precluded and the animals were of necessity instantaneously killed without the administration of any anodyne. They were at first killed with a blow of a weighted pipe, but later, it was found much simpler to fire a small calibre bullet directly into the medullary centers. The ureters were immediately removed and placed in the chilled physiological solution with as little handling or mechanical insult as possible. A small piece of kidney was clipped off with its adjacent ureteral pelvis and a small piece of bladder likewise, in order that the position of the segment might be readily recognized whether cutting off from the proximal or distal end.

Relevant to the extremities of the ureter, there is some controversy as to the comparative activity of various levels. Our experience as regards spontaneous contractions varied sufficiently. We concur with Gruber that "a perfect metabolic gradient from kidney to urinary bladder is not observed in the ureter." The point may be taken that Gruber worked with the entire ureter whereas we used only logitudinal segments and a few rings, and that trauma incident to cutting and securing to the apparatus might alter the irritability of a given segment.

So many were the factors modifying the spontaneous activity of the isolated ureteral segments that we feel loathe to propound any dictum at this time, but our experience in general inclines us to believe that distal segments contract spontaneously more often than do proximal segments, and are more irritable. Gruber stated that contractions are always

more forceful after a long period of rest, a fact which we noted on numerous occasions.

It was likewise noted by us that the ureters of pregnant animals are far less active and irritable. The ureters of nongravid female specimens were found to be most consistently dependable as regard spontaneous activity and irritability. When excising the ureters, inspection of the ovaries was routinely performed.

Another controversial topic in the matter of isolated ureteral tissue is the relative activity of ring, longitudinal segment, and the longitudinal strips preparations. Macht was of the opinion that the longitudinal strips exhibit a much lower response to epinephrine stimulation than do the rings, attributing the discrepancy to "lack of spontaneous peristaltic contractions in the longitudinal muscles." Gruber's findings do not agree with those of Macht. He (Gruber) claims that the longitudinal segments of isolated ureters are extremely rhythmical. In his paper, he illustrates by means of a tracing of longitudinal muscular contractions the fact that these contractions are responsible for the propulsion of fluids within the lumen of the ureter. Roth in his discussion of the activity of ureteral segments observes that the contractions of the proximal third of the ureteral, while of greater frequency, are smaller, and vice versa in the distal third. He cites the theory that the small muscular contractions are caused by circular muscle fibers and that the segment preparations give this type of reaction because of these circular fibers. He explained elongation of the ureter as due to contraction of the circular muscles and postulated this phenomenon to be the cause of relaxation occurring in longitudinal segments.

Our experience causes us to believe that the contractions of ring and longitudinal segments cannot be sharply differentiated and independently studied; that they react similary to the same modifying factors (i.e., stimulant drugs, spasmolytic drugs, or thermal changes); that the innervation of both layers of muscles is the same, and that a study of longitudinal preparations as regards drug action is quite as significant as that of the ring. Longitudinal segments, therefore were used

altogether after preliminary trials were conducted with the use of ring preparations.

In concluding this digression regarding the comparative experimental value of longitudinal and circular ring segments, it is of interest to note that Trattner observed in intact human ureters that there are two distinct stypes of waves: one, a small segmental or pendular movement and the other a propulsive or peristaltic wave. If we attempt to reconcile the ideas of Roth and Gruber with the notions of Trattner, it would appear that the longitudinal muscles perhaps are the most important from a practical standpoint and experimentally should be the preparation of choice in studying the action of drugs on the ureter.

The segments were kept from ten minutes to eight hours before use since other workers have shown that ureteral tissue exhibits spontaneous contractions from one to twenty-four hours after removal. Gruber reports spontaneous peristaltic and anti-peristaltic contractions in longitudinal segments as long as one hundred eight hours after removal. They were then attached to the oxygen tubes and gravity levers by means of linen thread and placed in modified Locke-Ringer urea solution in the constant temperature bath.

Oxygen was bubbled through each capillary tube (app. 2 mm. diam. lumen) at a rate of about 4–5 bubbles per second. Our experience confirms that of Stern and Macht to the effect that efficient oxygenation favors ureteral activity, whereas anoxia diminishes it.

The levers were weighted and adjusted in such a manner that the slightest contraction would be recorded, but also that all "slack was removed from the linen thread and the segment itself." Automatic timers were placed to write below each lever, for convenience in reading results. The speed of the kymograph drum used was 2.5 mm. per minute.

Results

The results which we present are based on 558 experiments using longitudinal segments of the dog ureter. We feel that this represents a rather comprehensive survey of the subject and that we have a sound basis for our conclusions.

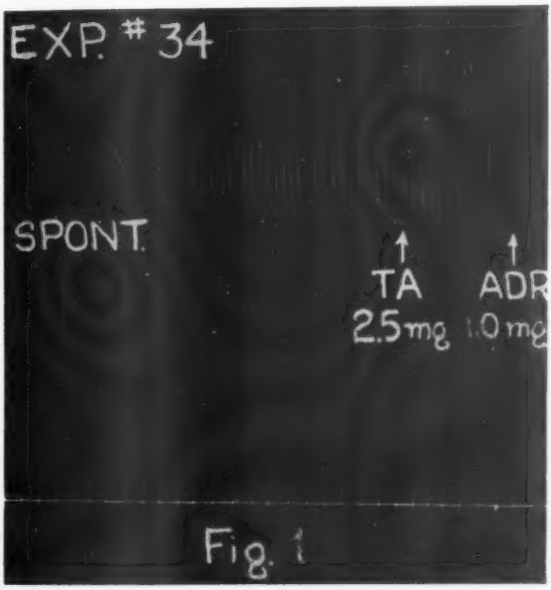

Fig. 1. The effect of TA on spontaneous contractions. After TA, twice the usual dose of ADR is ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

For sake of clarity as well as brevity, the main objectives reached in this study are summarized in the tables and figures which follow. The tables have been compiled from the figures and we consider that the tracings which make up the figures illustrate representative results for that particular drug or drugs in question. The experimental results elaborated herein are divided arbitrarily into three separate headings: Firstly, the effect of anti-spasmodics on spontaneously contracting ureteral segments; secondly, the effect of anti-spas-

modics on spontaneously contracting segments which have been stimulated to contractions by various drugs, and thirdly, the effect of anti-spasmodics on quiescent segments which have had contractions initiated by various stimulants.

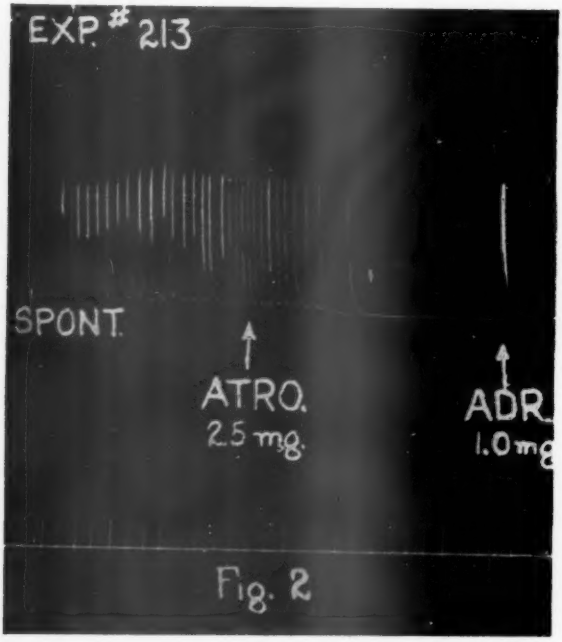

Fig. 2. The effect of ATRO on spontaneous contractions. After ATRO, twice the usual dose of ADR "broke through." Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

1. The actions of anti-spasmodics on spontaneous contractions are naturally of great physiological and clinical importance. The data which we offer show that "trasentin A" is clearly more effective than the other spasmolytics studied.

A typical tracing, as in Fig. 1, shows that this drug is swift and certain in decreasing the activity of the contracting ureter. The effect is promptly produced and lasts long. We have run

experiments for an hour after the administration of "trasentin A" and no further contractions were observed. Further, the most powerful stimulant, epinephrine, does not cause contractions once they have been stopped by "trasentin A." This is an important fact from a clinical standpoint and we will show that other anti-spasmodics do not possess this action.

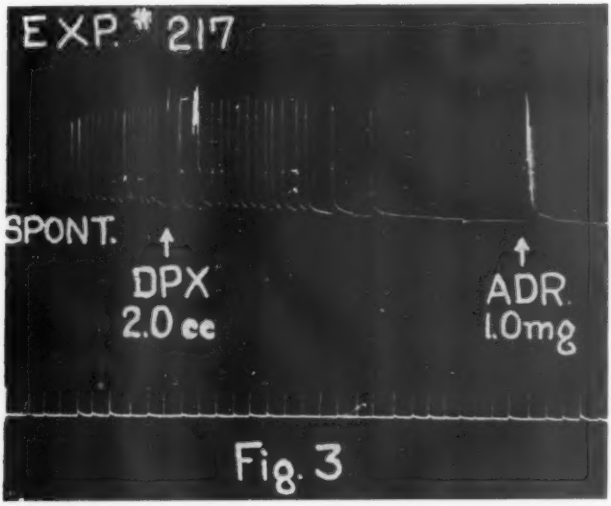

Fig. 3. The effect of DPX on spontaneous contractions. After DPX, twice the usual dose of ADR "broke through." Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

Since atropine is known to be an anti-cholinergic drug and also to possess some direct muscular action (Sollmann), a tracing as shown in Fig. 2, demonstrates two very salient features. The action as compared with "trasentin A" is slow in onset. While in Fig. 2, the time required for complete cessation is only 1 minute longer than that of "trasentin A" in Fig. 1, atropine is very inconsistent in this effect. This will be particularly noted in Fig. 7, where atropine was found to be ineffective in 20 minutes. Secondly, it is always an easy

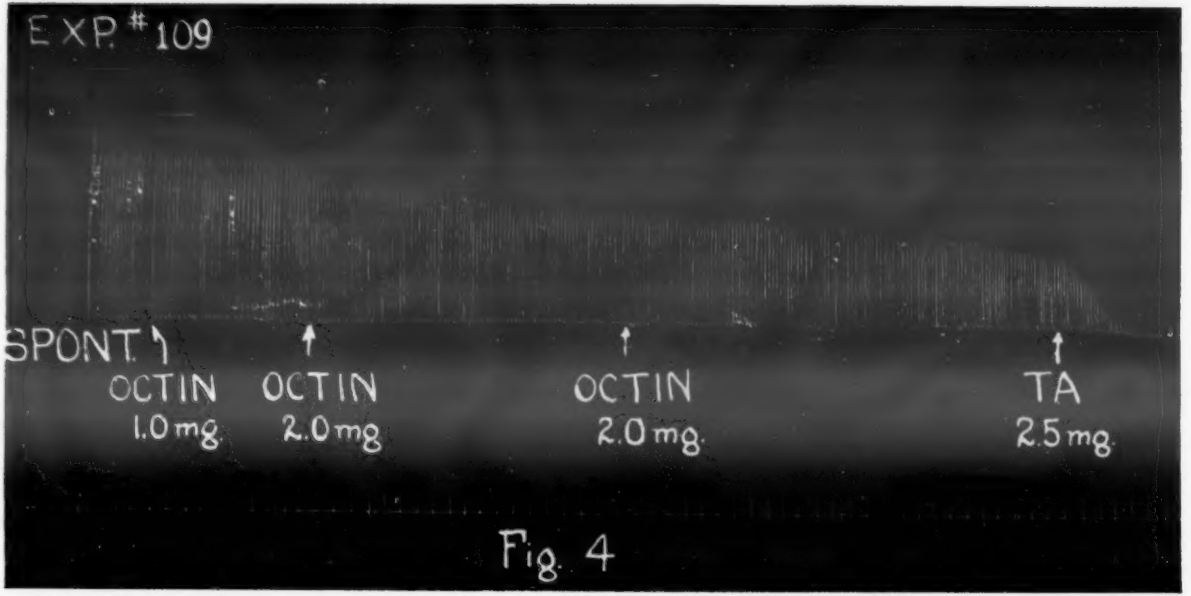

Fig. 4. The comparative effect of repeated doses of OCTIN and TA on spontaneous contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

matter to "break through" the anti-spasmodic effect of atropine by giving 1 cc. of epinephrine following the cessation of all contractions. We have never been able to accomplish this following "trasentin A." In other instances after atropine had for a few minutes apparently stopped the contractions initiated by epinephrine, typical epinephrine spasms later "broke through," as demonstrated in Fig. 22A. We can readily say that this has never been noted when "trasentin A" has been used to stop epinephrine contractions.

Another anti-spasmodic which has been heralded clinically as very effective is the deproteinated pancreatic extract, "depropanex" (DPX). Lazarus states that DPX is effective in relaxing and in causing contractions of the ureter to subside. Naturally, we do not think that our experimental results should be more contributory to the knowledge of this drug on the ureter; nevertheless, though opposed to those of Lazarus', they have been consistent and we feel that the tracing which he shows on DPX is far from conclusive. In Fig. 3, we see the most effective anti-spasmodic action of DPX which we obtained in 40 or 50 experiments. It is noteworthy that even here the time for action is 12 minutes and that preceding any anti-spasmodic effect there were several spasms produced by this drug. Furthermore, like atropine, "adrenalin" produces its characteristic type of contraction after DPX. That this is true is another reason for our casting some doubt on the opinions of Lazarus. Elliot and Nazum claim that on the blood pressure "DPX acts chiefly by counter-acting the action of epinephrine." We are not prepared to say whether DPX antagonizes adrenalin on the blood pressure and not on the ureter; but we do state that in these extensive experiments, DPX has not exhibited the property of counteracting adrenergic drugs.

It was noted that on several occasions that the administration of DPX to a quiescent ureter was followed immediately by vigorous contractions. Inasmuch as this effect, whether or not dependent upon the action of DPX, was scarcely compatible with the objectives of our investigation, the experiment was not repeated more than four or five times.

In many instances, DPX caused contractions of a spontaneously active ureter to increase in amplitude and rate over a period of sixteen minutes.

Methylamino-methyl heptene ("octin") has been shown to possess an anti-spasmodic action of the alimentary tract (Gruber, 1936; Jackson). Issekutz moreover claims that "octin" decreases the intestinal peristalsis of the cat which has been increased with physostigmine. With this in mind, it was suggested that we investigate the possible anti-spasmodic action of this ureter. We felt from the first that it would probably not be efficient on the ureter and that such is the case is shown in Fig. 4. Here we note that 3 doses of octin only progressively decrease the amplitude of contractions in contrast to "trasentin A" which was as usual very effective in its action. Possibly some of the action as seen here with "octin" may be caused by an ergotoxine-like action.

Van Duzen was one of the first to suggest that "theelin" counteracts ureteral spasm and others (Traut and McLane) have suggested that when certain estrogenic substances are most concentrated, the ureter is often times dilated. Our results, in these experiments using "emmenin" (the estrogenic placental hormone) containing 30 day-oral Coppil units per cc., are not wholly conclusive but they are suggestive of the validity of this opinion. We noted that when "emmenin" is added, as in Fig. 5, a spontaneously contracting segment showing periodic spasms responded by having its rate markedly decreased in 20 minutes. However, as in the case of "octin" and DPX, epinephrine produces its characteristic response. We felt that the 15 per cent alcohol which "emmenin" contains as a preservative might obscure some of the experimental evidence as to the efficacy of the preparation of "emmenin." However, a dose of alcohol identical to that contained in a dose of "emmenin" produced no apparent action attributable to the alcohol in 12 minutes, as shown in Fig. 6. When "emmenin" itself was added, there was complete cessation in 8 minutes. This effect of 15 per cent alcohol may be compared with observations in which, following adrenalin, it was found to reduce the height of contractions in 5 minutes. On the other hand, 7 minutes later, "emmenin" itself de-

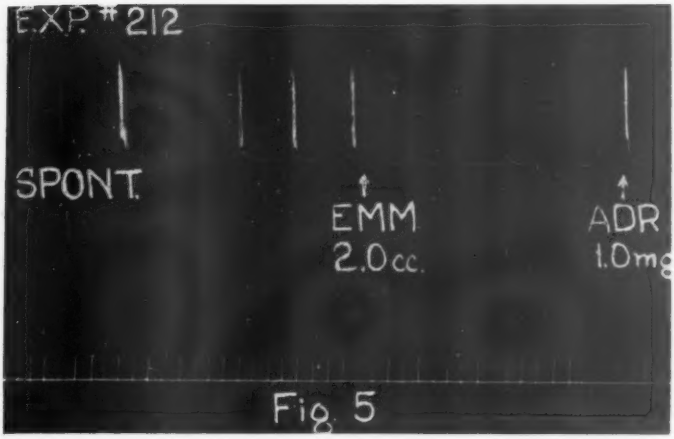

Fig. 5. The effect of EMM on spontaneous contractions. After EMM, twice the usual dose of ADR caused return of typical spasms. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

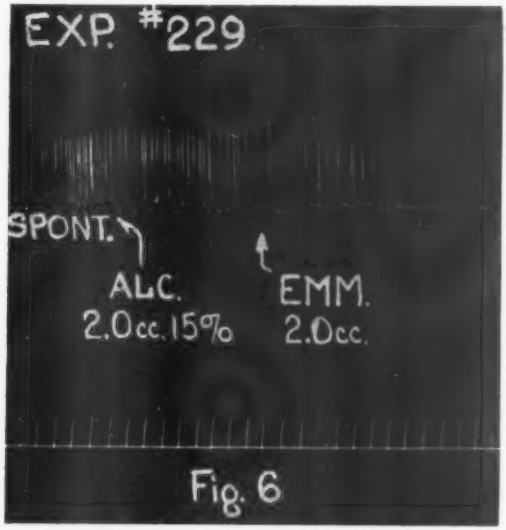

Fig. 6. The comparative effect of 15% ALC and EMM on spontaneous contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

creased the rate of contractions by ½ and the height of contractions by 2/3 in a 10-minute interval. We feel from other such experiments that "emmenin," in its own right, has some anti-spasmodic action. We hope to try an estrogen in aqueous solution which contains no alcohol.

Fig. 7. The comparative effect of EMM and ATRO on spontaneous contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

Another reason for our belief that "emmenin" inherently possesses the ability to bring about cessation of contractions is shown by an experiment in which the first dose of "emmenin" was without effect, while a second dose of "emmenin," after atropine (in itself ineffective) manifested a distinctly anti-spasmodic action, accomplishing complete cessation of contractions in 3 minutes (Fig. 7).

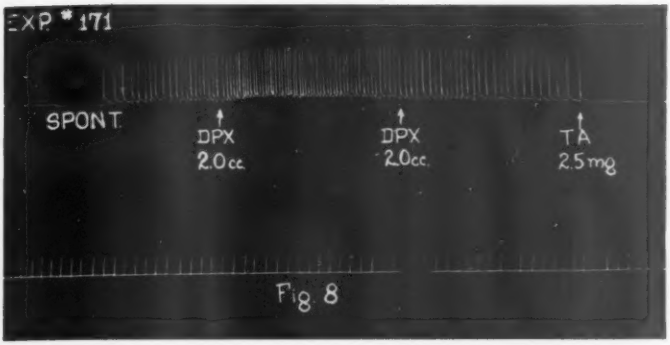

Fig. 8. The comparative effect of repeated doses of DPX and TA on spontaneous contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

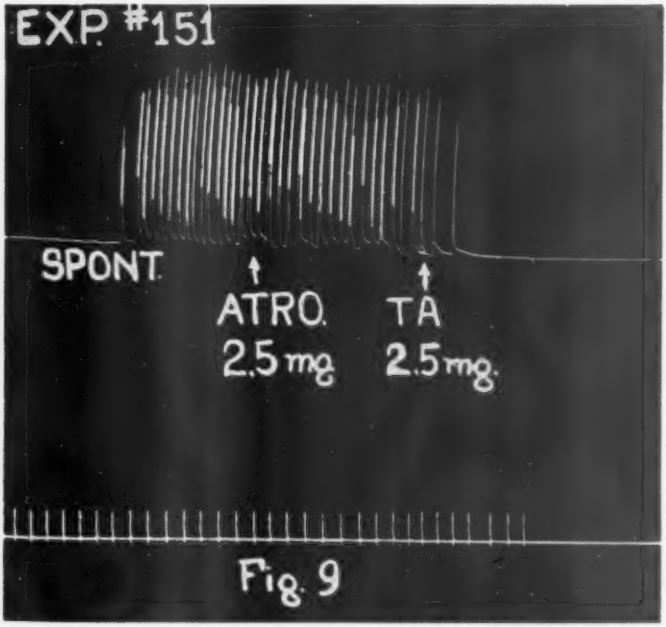

Fig. 9. The comparative effect of ATRO and TA on spontaneous contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

To emphasize further the effect of "trasentin A" as compared with other anti-spasmodics, we found that when DPX or atropine fails to act, "trasentin A" exhibits its usual efficacy (Figs. 8, 9).

Fig. 10. The effect of TA on spontaneous contractions stimulated by PROS. After TA, a large dose of PROS was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

2. The effect of anti-spasmodics on spontaneously contracting segments stimulated by various drugs may be illustrated by a few selected experiments. The action of "trasentin A" following prostigmine is to bring about a very prompt cessation of contractions, as shown in Fig. 10. This indicates the efficacy of "trasentin A" in antogonizing known cholinergic drugs.

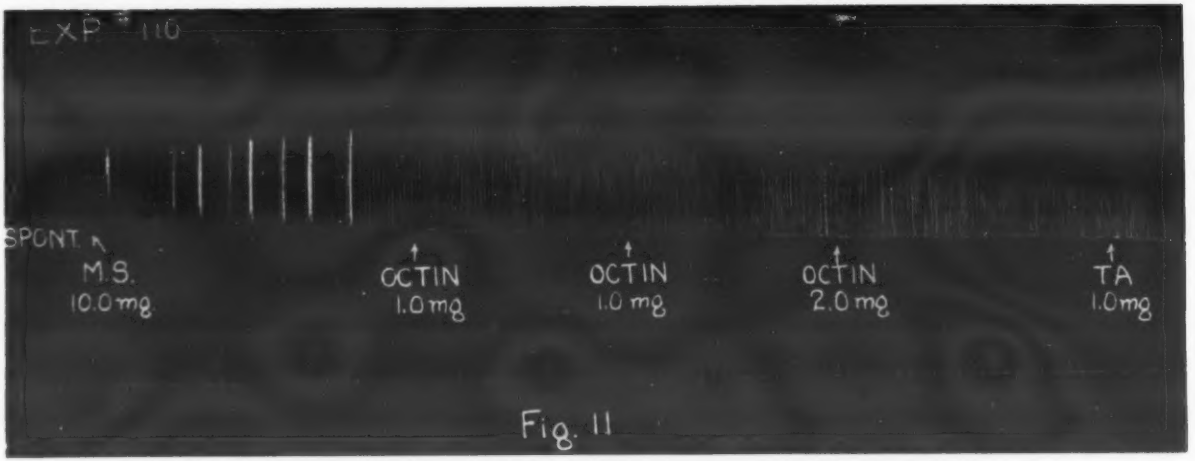

Fig. 11. The comparative effect of repeated doses of OCTIN and TA on spontaneous contractions stimulated by M.S. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

One of us (D.S.) has shown that morphine is probably cholinergic in many of its actions and Meier has shown that "trasentin A" is anti-cholinergic. Confirmation is afforded by the finding that after three doses of "octin" had failed to stop

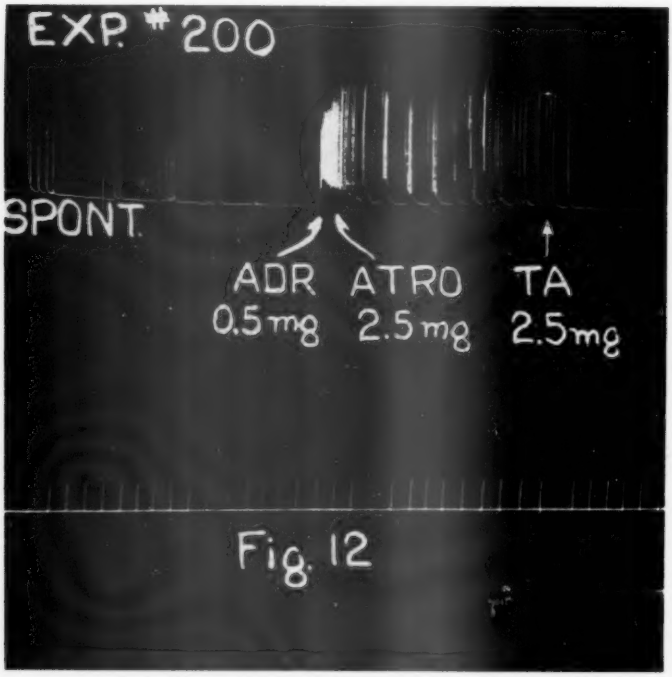

Fig. 12. The comparative effect of ATRO and TA on spontaneous contractions stimulated by ADR. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

spontaneous contractions enhanced by morphine, 0.4 of the standard dose of "trasentin A" quickly brought about cessation of contractions (Fig. 11).

It may be shown that atropine, even though it possesses some degree of muscular depression, does not satisfactorily antagonize epinephrine in stimulating ureteral contractions. On the other hand, "trasentin A" promptly relaxes the

epinephrine stimulated ureter, as indicated in Fig. 12. This demonstrates that "trasentin A" exhibits a marked anti-muscular, as well as an anti-cholinergic, anti-spasmodic effect.

In studying the effect of anti-spasmodics on quiescent segments which have been made to contract by various drugs,

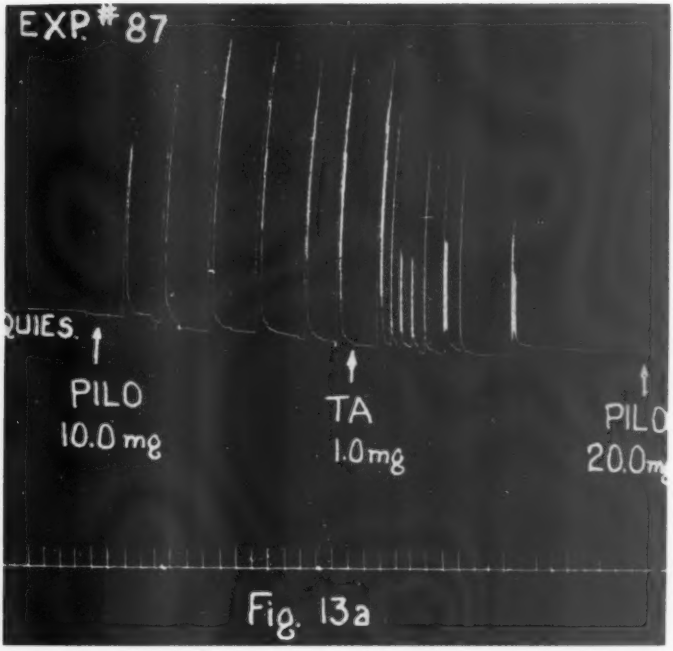

Fig. 13a. The effect of a small dose of TA on a quiescent segment after PILO had initiated contractions. After TA, a large dose of PILO was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

we found that the comparative actions of the anti-spasmodics are the same as in the case of spontaneously contracting ureters.

Fig. 13 clearly shows the antagonism of "trasentin A" for pilocarpine. In each instance the effect is almost instantaneous as regards decrease in the height of contractions. When

1 mg. of "trasentin A" was used to counteract the effect of 10 mg. of pilocarpine, the time of action was 10 minutes. When 2 mg. of "trasentin A" were used against the same dose of pilocarpine, the time for action was 5 minutes. When a larger dose of "trasentin A" was used against the standard dose of pilocarpine, the time for action was still shorter.

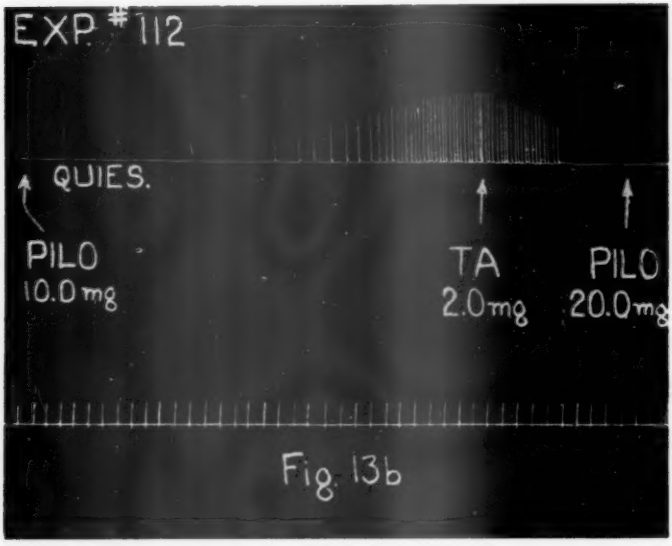

Fig. 13b. The effect of a sub-standard dose of TA on a quiescent segment after PILO had initiated contractions. After TA, a large dose of PILO was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

It seems that up to the optimal effect of "trasentin A" on pilocarpine the efficiency of the latter as an anti-spasmodic is inversely proportionate to the dose used. Table IV emphasizes the inverse efficiency proportion of decreasing doses of "trasentin A." If a graph is constructed by plotting the dose along one axis and the time of action along the other, it will be seen that most points fall on a straight line.

That "trasentin A" is equally effective on the ureter as an anti-spasmodic against other known cholinergic drugs is exemplified in Fig. 14, where physostigmine was used as the stimulant. The effect of "trasentin A" here is typical of its usual actions on all ureters whether spontaneously or contracting as a result of artificial stimulation.

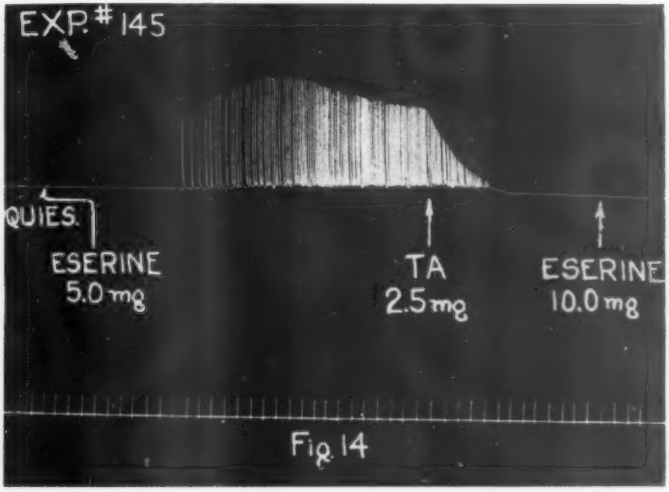

Fig. 14. The effect of TA on a quiescent segment after ESERINE had initiated contractions. After TA, twice the starting dose of ESERINE was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

Still another drug, morphine, which we believe to have a true cholinergic action, is antagonized by "trasentin A" in a manner similar to that with other known cholinergic drugs (Fig. 15b). Further to substantiate previous reports by Slaughter, we have shown that the ureter responds to the potentiating effect of morphine and prostigmine. Such an effect is noted in Fig. 16. In this instance, a marked increase of tonus was observed but a small dose of "trasentin A" (1 mg.) proved as effective as expected.

Fig. 17 represents the usual anti-spasmodic effect of atropine (a large dose) against the stimulant action of morphine. There was no cessation of contractions; in fact, they actually increased slightly in rate as compared with morphine. In 10 minutes after atropine had failed, "emmenin" was very effective. We have already mentioned this peculiar effect and it

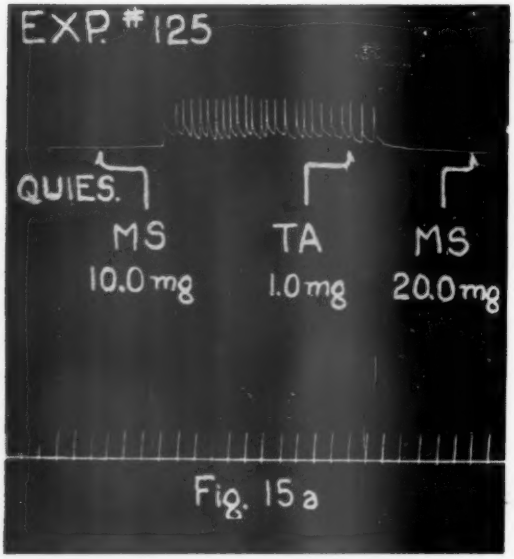

Fig. 15a. The effect of a small dose of TA on a quiescent segment after M.S. had initiated contractions. After TA, twice the starting dose of M.S. was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

occurs with such frequency that we believe there is a close relationship of anti-spasmodic actions when the two are used in this sequence. Fig. 18 more clearly demonstrates this reaction. Here after morphine stimulation, "emmenin" alone was ineffective and atropine in large doses was also ineffective but the second dose of "emmenin" after atropine proved very efficient. In this instance, twice the starting dose of morphine was ineffective after contractions ceased.

The tracing shown in Fig. 19, clearly demonstrates the superiority of "trasentin A" once again. Here after two doses of DPX had not only failed to stop the contractions due to morphine but had actually increased their height, "trasentin A" in the "standard" dosage was, as usual, very effective.

It may be argued that, while "trasentin A" seems very potent against spontaneously contracting ureters and those

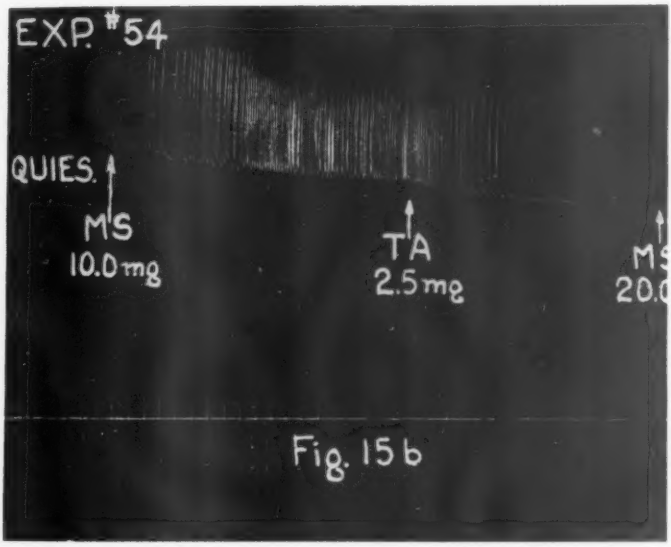

Fig. 15b. The effect of the usual dose of TA on a quiescent segment after M.S. had initiated contractions. After TA, twice the starting dose of M.S. was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

stimulated by cholinergic drugs, this action might be lacing when quiescent ureters are stimulated with known sympathetic drugs, since the ureter is believed to respond better to such drugs due to its stronger inherent, motor, sympathetic nerve supply. Our results, however, indicate that "trasentin A" is equally effective following both types of stimulants.

"Trasentin A" quite promptly antagonizes both the contractions and increased tonus due to epinephrine (Fig. 20),

and doubling the dose of epinephrine after cessation of movement was found to be ineffective. When "emmenin" is used against a sympathetically stimulated ureter, it is generally more effective than it is when the ureteral segment has been contracted by cholinergic drugs, as shown in Fig. 21a. We have spoken of the possibility that alcohol might play a part

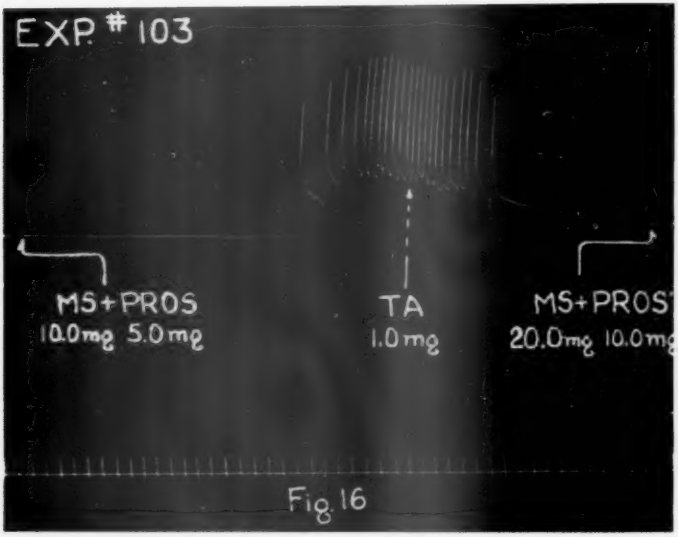

Fig. 16. The effect of a small dose of TA on a quiescent segment after M.S. plus PROS had initiated contractions. After TA, twice the starting dose of M.S. plus PROS was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

in the action of "emmenin" on ureteral segments. For sake of comparison, Fig. 21b again shows that when an equivalent amount of alcohol is compared with "emmenin," the latter seems clearly to possess real anti-spasmodic action. Since the "emmenin" here has been added 7 minutes after the alcohol, it might be that the alcohol in the "emmenin" simply added its effect to the "alcohol alone" action. We do not believe this is true, since, when we added two or three doses of 2 cc. of a 15 per cent solution, it did not appear effective.

Marrazzi has suggested that as regards sympathetic synaptic transmission, atropine does show an actual sympathetic antagonism. Fig. 22a shows the apparent muscular depression effect of atropine against epinephrine-stimulated contractions, and also illustrates the spontaneous "break through" of such contractions after apparent cessation has taken place. It is

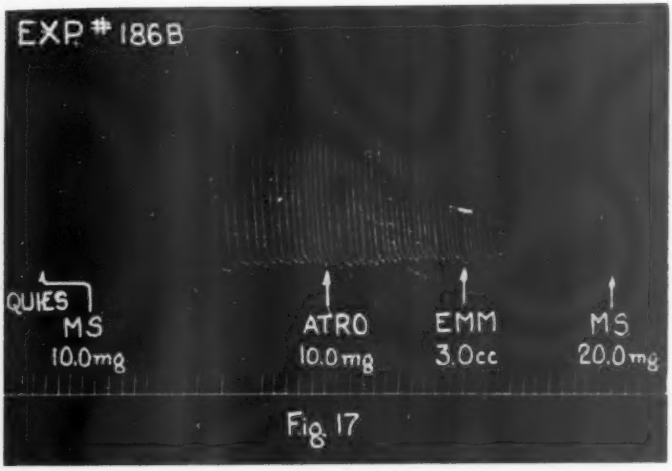

Fig. 17. The comparative effect of ATRO and EMM on a quiescent segment after M.S. had initiated contractions. After EMM, twice the starting dose of M.S. was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

important to note again, Fig. 22b, that after atropine had failed to alter appreciably the contractions, "emmenin" was effective. This illustrates once more the possibilities of "emmenin" action following the belladonna derivative.

Fig. 23 illustrates the features of "octin" action. It is interesting to note that even following epinephrine the first dose of "octin" caused increased activity of the ureteral segment and then a second dose of "octin" 9 minutes later was strikingly effective. We can only suggest that the action here might be an ergotoxine-like effect.

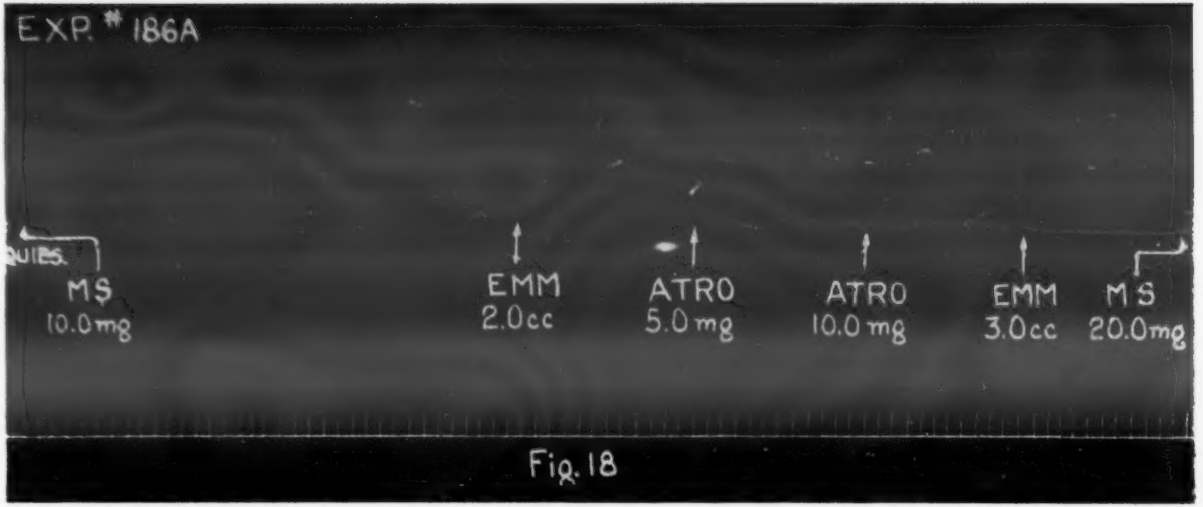

Fig. 18. The comparativē effects of repeated doses of ATRO and EMM on a quiescent segment after M.S. had initiated contractions. After EMM, twice the starting dose of M.S. was ine ffective. Kymograph speed = 2.5 mm/minute. Time trace = 1 minute.

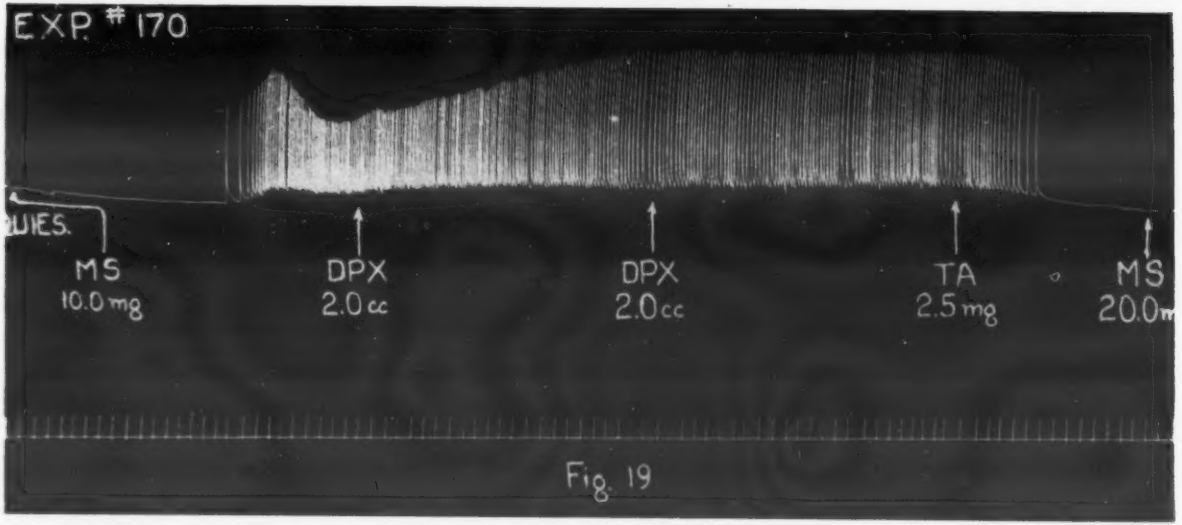

Fig. 19. The comparative effect of repeated doses of DPX and TA on a quiescent segment after M.S. had initiated contractions. After TA, twice the starting dose of M.S. was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

Fig. 24 demonstrates that even when adrenalin is given previously, DPX does not actually exhibit the anti-epinephrine property which Elliot states that it has on blood pressure. It is true, in this instance, after two doses of DPX, a definite action was noted, but it required the further addition of a large dose of atropine to bring about complete cessation of contractions. While in this particular experiment, a double starting dose of adrenalin was not used after all

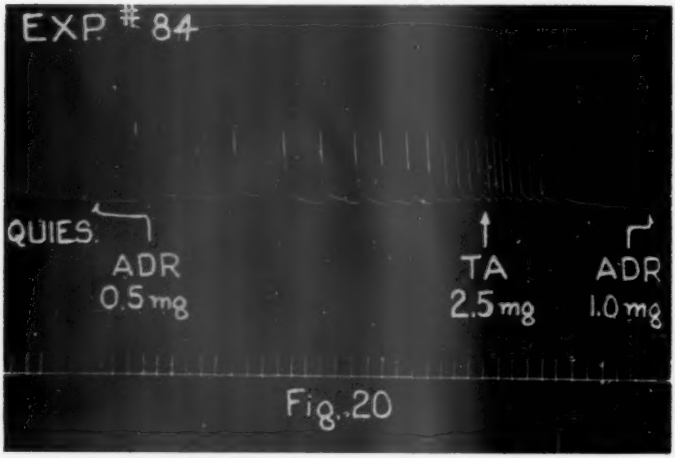

Fig. 20. The effect of TA on a quiescent segment after ADR had initiated contractions. After TA. twice the starting dose of ADR was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

contractions had ceased, we feel confident that such a starting dose would probably have re-initiated contractions.

Finally, we were able to show what we believe would be the optimal effect of DPX on an epinephrine-stimulated ureter. In Fig. 25, DPX temporarily causes the epinephrine initiated contractions to cease, but very shortly thereafter they "break through" and return to the original height exhibited before DPX administration. However, when the standard dose of "trasentin A" was administered, in contradistinction, the effect was almost instantaneous and it was permanent.

We have suggested above, for the sake of the argument, that "trasentin A" might not be effective against sympathetically stimulated contractions but we have proved beyond doubt that this anti-spasmodic is effective under such circumstances. Furthermore, as noted in Fig. 26, ephedrin, which

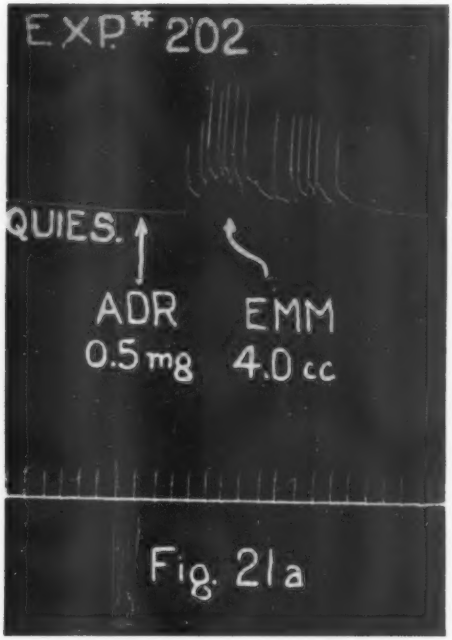

Fig. 21a. The effect of EMM on a quiescent segment after ADR had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

is both a known adrenergic and myonergic drug is antagonized by "trasentin A" equally as well as is adrenalin, which is as far as anyone knows, only adrenergic.

To complete the versatile anti-spasmodic action of "trasentin A," we note in Fig. 27 that "trasentin A" counteracts the action of a direct muscular stimulant, such as pituitrin.

Fig. 28 demonstrates that the human ureter responds to a typical contraction-producing drug, such as morphine sulfate,

as well as to a typical spasmolytic drug, "trasentin A." The reaction as noted here is comparable in every way to the results obtained on ureteral strips taken from the dog and would indicate that at least a goodly part of the results thus obtained can be transferred to those in man.

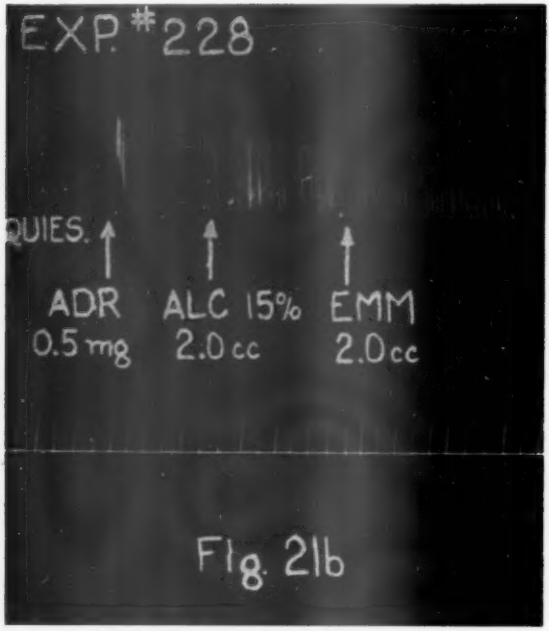

Fig. 21b. The comparative effect of 15% ALC and EMM on a quiescent segment after ADR had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

Discussion of Results

Even a cursory perusal of these results demonstrates an outstanding incontrovertible fact; i.e., "trasentin A" was the only anti-spasmodic used in these experiments which was strikingly effective in counteracting all types of ureteral contractions, be they spontaneous or elicited by cholinergic, adrenergic or myonergic drugs.

The actions of the drug on ureteral contractions may be summarized by stating that it is (1) anti-cholinergic because it is effective against prostigmine, physostigmine, pilocarpine and morphine; (2) anti-adrenergic because it is effective against adrenalin and ephredrine, and (3) anti-myonergic because it is effective against ephedrine and pituitrin.

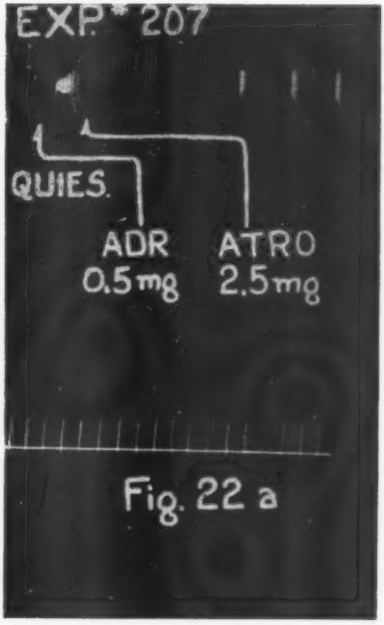

Fig. 22a. The effect of ATRO on a quiescent segment after ADR had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

There are some discrepancies in the degree of activity of the other anti-spasmodics when they are used on ureters stimulated by cholinergic or adrenergic drugs. The comparison which we present of the various anti-spasmodics reflects their optimal activity on the isolated ureter and as the results have shown, their best effect when compared with "trasentin A" was consistently inferior.

Trasentin (diphenyl-acetyl-diethylamino-ethanolester-hydrochloride) has had a wide clinical usage as an anti-spasmodic in various disorders; i.e., gastritis, gall bladder colic, spastic bladder, and other allied clinical entities. It was marketed both in tablet and ampule form but the latter is not extremely stable. For this reason, the phenylcylohexyl ester of acetic acid ("trasentin A") has been synthesized and to date has been shown to be very stable in solution. The pharma-

Fig. 22b. The comparative effect of ATRO and EMM on a quiescent segment after ADR had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

cology of this new spasmolytic drug has been carefully studied. Briefly, it has a more pronounced anti-spasmodic effect than trasentin, whether used on isolated intestine, intact intestine, against the motility of the stomach and gall bladder, or on the isolated uretus. Further, "trasentin A" possesses a spasmolytic action against para-sympathetic stimulation or direct muscular stimulation.

Intravenous doses of this drug do not have a deleterious effect on the blood pressure or respiration. We noted that

in the dog and cat, "trasentin A" in proper dosage actually stimulates respiration in most instances following morphine depression. It has also been pointed out that "trasentin A" is less toxic than the old trasentin and experimentally doses required to counteract smooth muscle stimulation are usually less than those needed for trasentin.

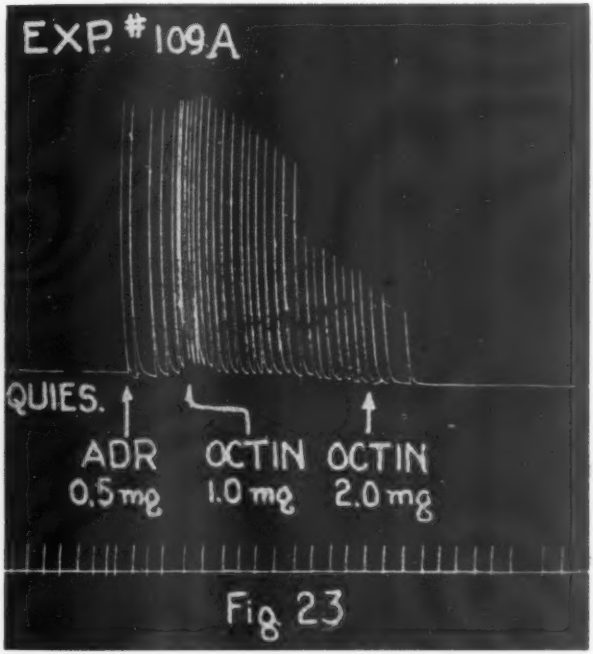

Fig. 23. The effect of repeated doses of OCTIN on a quiescent segment after ADR had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

We have been specifically concerned with the effects of "trasentin A" in this problem as regards the contraction of the ureter. Our experiences have shown that it is a very powerful spasmolytic drug. The usual effective dose against any type of ureteral contraction (spontaneous or otherwise)

is 2.5 mg. in a 100 cc. bath. This was the "standard" dose used. As compared with time of action, "trasentin A" was extremely rapid; i.e., 2–5 minutes after administration. This average time was much lower than with the other spasmolytic drugs. On occasion when the strength of the contracting ureter was about 2 plus, smaller doses of "trasentin A" sufficed.

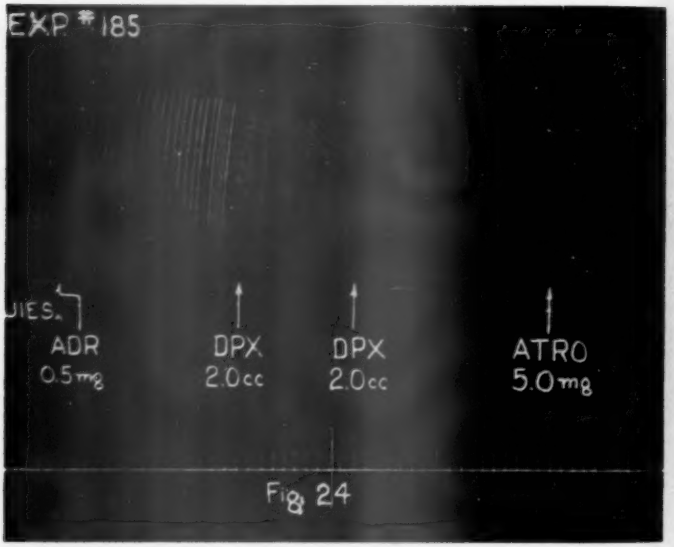

Fig. 24. The comparative effect of repeated doses of DPX and ATRO on a quiescent segment after ADR had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

It is important to note that these results show that "trasentin A" is always effective while the other anti-spasmodic drugs are extremely variable in their efficiency. Furthermore, this drug had much more permanency in its action and it is extremely difficult, if at all possible, to restimulate a ureteral segment once it had been stopped by "trasentin A." With the other anti-spasmodics the reverse is true. It should be pointed out here that whether the tracings shown in the text are so labelled, in most instances, twice the stim-

ulating dose of the stimulant was used following the cessation of contractions.

We have consistently noted that this spasmolytic drug is more effective against neurogenic stimulants than against a direct smooth muscle stimulant such as pituitrin. We believe this is of some clinical importance since, when in ureteral colic or any spasm of the ureter morphine is given for pain,

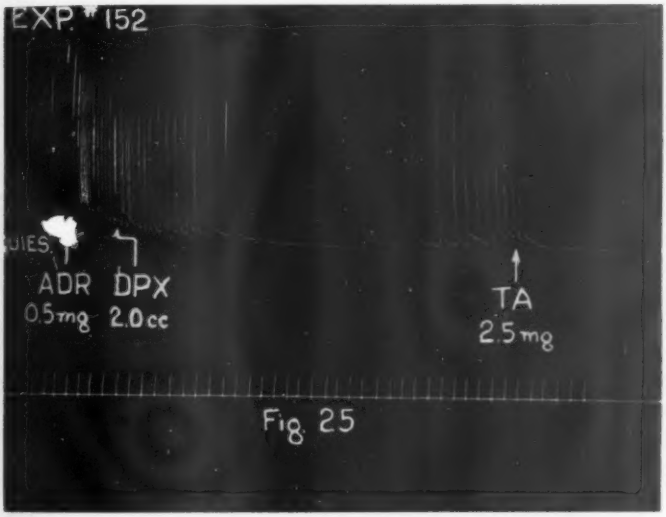

Fig. 25. The comparative effect of DPX and TA on a quiescent segment after ADR had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

it unquestionably increases the contractions of the ureter. However, if "trasentin A" is given following the morphine, it should suppress the increased activity of the ureter due to the original spasmodic condition.

Since "trasentin A" has been shown to be the most efficient spasmolytic drug, both in speed and permanency of action, we feel that it is proper to suggest that clinical usage of it should give desired results. Preferably, however, it should not be administered intravenously.

In discussing the effects which we obtained with atropine, the tracings clearly indicate that even though "trasentin A" is supposed to owe part of its action to an atropine-like effect, atropine itself is not nearly as efficient as "trasentin A." This is demonstrated time and time again when it is noted that after atropine fails to act, "trasentin A" is very efficient. We are confident that this lack of effect is not due to an insuffi-

Fig. 26. The comparative effect of DPX and TA on a quiescent segment after ephedrine had initiated contractions. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

cient dose of atropine. These results of atropine are somewhat at variance with those of Macht, who stated that large (5–10 mg.) doses are relaxing while small doses were stimulating in their effect. Briefly, we do not feel that atropine is consistently effective as a spasmolytic drug on the ureter, but in all fairness, we should say that against cholinergic drugs and on all occasions against spontaneously contracting segments, it seems to be fairly efficient. Naturally, one would not expect atropine directly to antagonize stimulation caused by adrenalin, which is an adrenergic stimulant, while atro-

pine is chiefly an anti-cholinergic drug. It has been stated (Sollmann) that atropine does possess some direct muscular depression and in a few instances where atropine seemed to be active it was probably due to this direct muscular action.

As regards DPX, our experiences with it have not been heartening. According to Elliot, this pancreatic extract has as

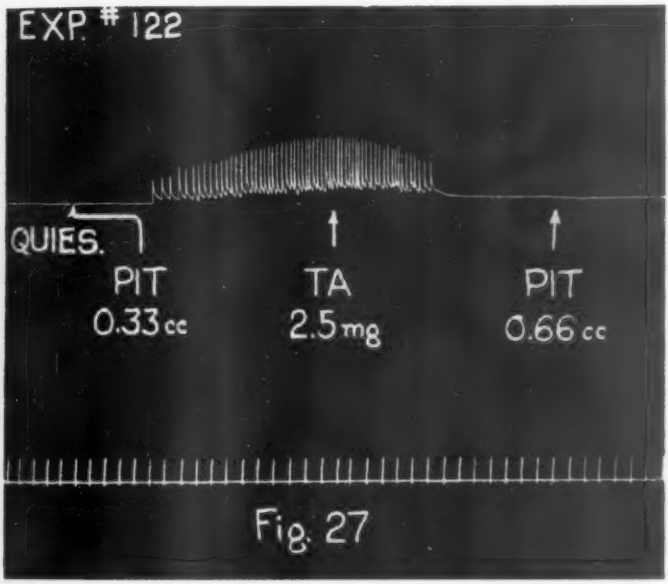

Fig. 27. The effect of TA on a quiescent segment after PIT had initiated contractions. After TA, twice the starting dose of PIT was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

one of its chief actions, a direct antagonism towards adrenalin. In our hands, this has not proven to be the case. In fact, on many occasions, as will be noted in the tracings, DPX actually causes an increased number of contractions following the administration of adrenalin which had previously initiated them. In a few experiments, DPX is effective after large doses. However, we feel that the doses used in these instances are much greater than comparable therapeutic doses

would be. We believe that Villaret was perhaps right when he suggests that many of the actions of deproteinated pancreatic extracts are due to its choline and histamine content even though it is very small. Should we accept this hypothesis, it might explain the action on the ureter which we usually ob-

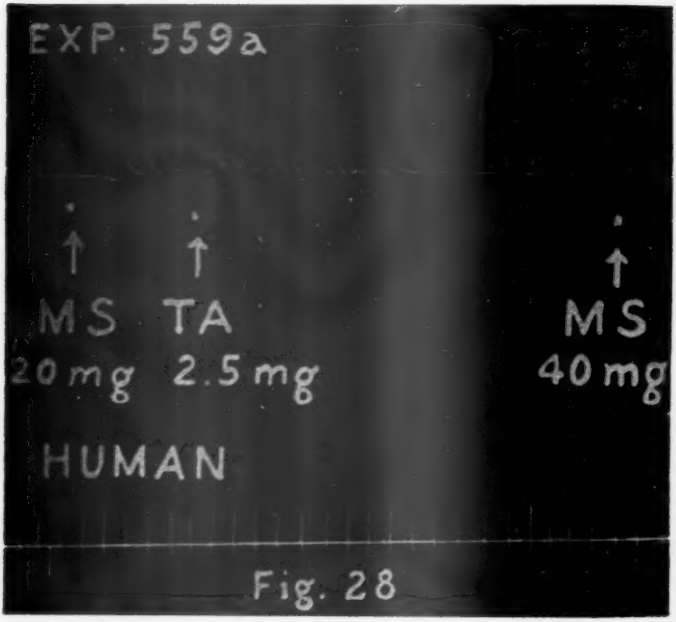

Fig. 28. The effect of TA on a quiescent HUMAN segment after M.S. had initiated contractions. After TA, twice the starting dose of M.S. was ineffective. Kymograph speed = 2.5 mm/minute. Time Trace = 1 minute.

tained. It is interesting regarding this spasmolytic drug that Elliot states that it must be given intravenously to be effective, while Carroll uses subcutaneous injections.

We were not surprised as we have mentioned previously, that octin is usually ineffective as an anti-spasmodic on the ureter. Gruber, Jackson, and Issekutz independently have shown it to approximate in action a sympathetic stimulant.

Such an effect is advantageous in decreasing the activity of the gastro-intestinal tract. However, octin's action on the ureter would be that of stimulation. This is clearly shown in many instances. In rare occasions when there seems to be a spasmolytic effect of octin, we can only attribute it to the possible ergotoxine-like action due to this drug.

The possibility of an estrogenic substance possessing a spasmolytic effect on the ureter is of clinical importance. Van Duzen called attention to the clinical effectiveness of "theelin," particularly when it follows atropine or belladonna. We have noted consistently that the substance we used was always much more anti-spasmodic when it was preceded by atropine. Further, Jenkins and Van Wagenen have shown that the placental extracts in monkeys cause marked dilation of the ureters. Traut et al. state that in women the ureters are markedly dilated during pregnancy, and Langworthy and Brack using rabbits noted a marked increase in bladder capacity during the later stages of pregnancy. The last workers believe that "progestin" is the female sex hormone responsible for the bladder dilation and state that "estrin" improves the condition. They further argue that the same process is responsible for the ureteral dilation as described by Traut et al.

Langworthy's work is opposed to that of Jenkins and Van Wagenen. Since the latter state definitely that in monkeys the placental hormone is responsible for ureteral dilatation, and since the estrogenic hormone in pregnancy comes chiefly from the placenta, and since it assumes an ascendency in influence as pregnancy progresses as compared to progestion, we can only conclude that Jenkins is referring to a placental estrogenic hormone as the cause of ureteral widening in the monkey. Since we used a placental estrogenic hormone, emmenin, and found it to possess spasmolytic activity on the ureter, we feel that our work substantiates the experiences of Jenkins and not that of Langworthy. Van Duzen used "theelin" clinically, and found it to be effective against certain types of increased ureteral activity. We are not in a position at this time to state definitely the exact correlation between various estrogenic substances as they affect the

ureter, but we are convinced that the placental estrogenic hormone does possess certain spasmolytic properties on this organ. To add weight to these conclusions, we noted during the course of these experiments, that ureters taken from pregnant female dogs are not easily stimulated by any of the drugs used for this purpose. Unfortunately, we were unable at the time of these experiments to obtain an aqueous estrogenic preparation. As stated before, the emmenin which we used contained 15 per cent of alcohol. This amounted to 0.003 cc. of alcohol in the entire bath. In order to compare the effect which the alcohol in the estrogenic preparation might possess, we added an equivalent amount of straight alcohol. We do not feel from the tracings shown that alcohol itself was the sole cause of the spasmolytic property of emmenin, if indeed it had an effect at all. It is true that Gruber pointed out that a hypnotic, phenobarbital, does possess anti-spasmodic properties. He used 50–100 mg. of phenobarbital in a 110 cc. bath. This dose caused a decreased height of contraction. Doses of alcohol needed for general hypnotic effect are much greater than phenobarbital and the dose Gruber used is 16–33 times the amount of total alcohol which we administered when we used emmenin. We feel, therefore, from the clinical and experimental evidence that emmenin does possess a certain amount of anti-spasmodic effect on the ureter.

Papaverine was not compared with other spasmoyltic drugs in this project since use of it clinically has fallen into disrepute.

We have corroborated the finding of Macht and Gruber in relation to morphine and epinephrine. We have demonstrated with a greater degree of clarity the effect of such stimulating drugs as physostigmine and pilocarpine. Roth in his work on the ureter, was not able to obtain consistently good effects with pilocarpine (if at all) and Macht, on the other hand, does not show clear-cut tracings to illustrate the stimulant property of physostigmine. We have demonstrated the cholinergic property of prostigmine and have shown as in many other instances that morphine is potentiated in its action on the ureter by prostigmine. In regard to stimulant drugs, ephedrine was shown to have the property of causing the

ureter to contract with increasing force. This is of importance since we have pointed out that ephedrine does not have a noticeable adrenergic effect on the bladder.

We have shown for the first time that pituitrin, a smooth muscle stimulant, stimulates excised ureteral muscle.

Finally, we have added to the pharmacological evidence already presented by other workers that the ureter has a double innervation: sympathetic and parasympathetic. This is of great importance as far as the ureter is concerned because both innervations appear to be motor rather than to oppose one another in activity.

Clinical Comment

The clinical evaluation of the efficiency of various drugs affecting the human ureter has been most difficult. This is readily appreciated when one considers the multiplicity of drugs recommended for aiding the passage of an ureteral stone. The administration of morphine in uretero-lithiasis has long been routine practice, on a purely empirical basis, yet it is now known that this drug is actually stimulant to the ureteral musculature. On the contrary, papaverine is an ureteral muscle spasmolytic. Here is raised the question as to what precise effect is desired. Again, another question is posed: should there not be cognizance taken of the difference between progressive peristaltic activity of the musculature and spastic contraction thereof? Is it rational to administer a muscle stimulant in order to force the calculus onward by hydraulic or peristaltic pressure, or should there be used some agent which will cause relaxation of the ureteral wall permitting urine to pass the calculus, and release the "grip" on the ureteral wall on the calculus? It is our impression that urologists are not in accord in answering this question.

What is the effect of an ureteral stimulant as used in a case of ureteral calculus? Firstly, there is contraction of ureteral musculature, with a resultant increase in the intra-ureteric pressure. Whether or not it causes the ureteral wall to grasp the calculus more firmly is debatable; but if the calculus is impacted will there be passage of urine about the

calculus only when the ureteral musculature thusly becomes exhausted?

Could this exigency be regarded as analogous to the dilatation of the cervix uteri by the oncoming head? In the latter case it is necessary that the cervix be dilated or that one of several formidable operative procedures (incision of the cervix, or Caesarean section) be performed. Is the same problem involved in the instance of the impacted ureteral calculus? If the cervix uteri is relaxed by sedatives, the muscles of the fundus share in the relaxation, thus obliterating the principal expulsive force. In the case of the ureter, relaxation of the muscular need not destroy the expulsive force entirely. The kidney is constantly elaborating urine unless the intrapelvic pressure rises above a certain level (40 mm., Hg) at which point secretion slows in rate or ceases entirely. When the ureteral wall is relaxed, urine may escape, thus lowering intrapelvic pressure. Unfortunately, however, this is not always possible. With reduction in intrapelvic pressure renal function is again resumed, and the intraureteric pressure is again raised unless the urine escapes into the bladder. Of course, there may be some escape of the urine around the stone, and moderate stimulation may actually assist its downward movement. This, however, is not invariably the rule, and there exist no reliable criteria as to whether a stimulant may alleviate or aggravate the renal stasis.

Another source of controversy lies in the older concepts concerning sympathetic and parasympathetic nerves and the antagonism existing between them. It has been demonstrated in the present work that epinephrine enhances (rather than inhibits) the ureteral muscle contractions instigated by cholinergic drug. It is felt that the older theories regarding antagonism are responsible for the suggestion of octin as an ureteral anti-spasmodic. Octin is adrenergic in nature. In the intestinal tract where the sympathetic nerves are generally antagonistic to the parasympathetic fibres, its action would be, understandably, anti-spasmodic. In the ureter this picture of antagonism cannot be demonstrated experimentally, and octin is not to be regarded as an ureteral spasmolytic.

Relevant to the controversy regarding the use of anti-spasmodic or stimulant drugs, it occurred to us that a rational approach to the problem might lie in a careful study of the effects of drugs of each type in the laboratory followed by correlation of the results obtained with those from clinical study. An attempt is now being made to evaluate clinical observations. By way of a preliminary report, it may be stated that the administration of trasentin or trasentin 6H in ureteral colic is followed by relief in most instances. In a previous paper we cautioned against the use of trasentin in combination with morphine. It is felt that morphine is responsible for slowing the respiration. Trasentin has been administered intravenously but the attendant side reactions (acute loss of sense of well being, vertigo, loss of orientation) were of such degree as to discourage routine use of this route of administration. Oral administration of the drug in cases of dull "renal" pain due to the ureteral spasm has proved very effectual. It is felt that the addition of some rapidly acting sedative which does not cause muscle contraction would be very desirable in many cases. However, we would not hesitate to use it in conjunction with morphine intramuscularly if circumstances demanded. Trasentin is certainly better than atropine.

"DPX" has relieved post cystoscopic spasms at times, but at other times has failed. We have not used "octin" clinically. The laboratory results obtained with the use of "emmenin" are a source of considerable interest. We have previously reported our results with "theelin" but have not used "emmenin." We feel that it offers considerable promise especially in women in whom the hormone influence might be desired for additional actions other than on the ureteral muscle.

Summary

"Trasentin A" is the most efficient and consistent ureteral spasmolytic drug found in this study. "Trasentin A" has an anti-spasmodic effect on the isolated ureter, whether segments were spontaneously contracting or were stimulated by adrenergic, cholinergic, or myonergic drugs.

Atropine is fairly reliable in stopping the spontaneous contractions of ureteral segments and those stimulated by cholinergic drugs.

"Depropanex" is inconstant in its effects on the ureter; on occasion is actually causes stimulation. "Octin" usually stimulates the ureter. When "octin" stops contractions, it is believed to be due to an ergotoxin-like effect. "Emmenin," an estrogenic placental hormone, best approaches the efficacy of "trasentin A." It is efficient following atropine.

Certain new technical problems concerning the use of excised ureteral tissue have been discussed. Possible clinical applications of these results are described.

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TABLE I

Effect of Anti-Spasmodics on Spontaneous Contractions

TABLE I

Effect of Anti-Spasmodics on Spontaneous Contractions

Exp. No.	Fig. No.	Type of Contractions*			Effect of Anti-Spasmodics on Contractions				Comment
		No. per min.	Height in mm.	Strength 1 + to 4 +	Drug Used	Dose mg. or cc.	Tonus + or −	Time for Action Minutes†	
34	1	3	12	3+	TA	2.5	−1	5.0	1.0 mg ADR after TA—no effect.
213	2	3	19	3+	ATRO	2.5	−1	6.0	1.0 mg. ADR after ATRO—typical contractions.
217	3	3	12	3+	DPX	2.0		12.00	1.0 mg. ADR after DPX—typical contractions.
109	4	7	27	4+	OCTIN	1.0			−1 Height in 10 minutes.
		7	24	4+	OCTIN	2.0			−2 Height in 32 minutes.
		7	15	2+	OCTIN	2.0			−1 Height in 14 minutes.
		7	12	2+	TA	2.5	−1	5.0	
212	5	3	15	4+	EMM	2.0	−1		Rate decreased to 0.5 per min. in 19 min. 1.0 mg. ADR after EMM—typical contractions.
229	6	5	12	3+	ALC	2.0			No action in 9 minutes.
		5	12	3+	EMM	2	−4 (2 min.)	2.0	After cessation of contractions, strength 2 + for 5 minutes, then complete cessation.
187A	7	12	47	4+	EMM	3.0			No action in 9 minutes.
		12	47	4+	ATRO	10.0			No action in 20 minutes.
		12	47	4+	EMM	5.0	−2	3.0	After Atropine, Emmenin seems effective.
171	8	3	11	3+	DPX	2.0	+1		No action in 17 minutes. Rate incr. to 6 per min.
		3	11	3+	DPX	2.0			Rate decreased to 1 per minute in 17 minutes.
		1	13	3+	TA	2.5	−1	0.5	Action instantaneous.
151	9	9	25	4+	ATRO	2.5	−0.5		
		6	23	4+	TA	2.5	−1.0	2.0	

*Data shown as of 3 minutes previous to next drug.
†Complete cessation of contractions.

STUDIES OF THE A-V NODE: IV. A CLINICAL STUDY OF ATRIOVENTRICULAR NODAL RHYTHM

ARTHUR RUSKIN, W. FRANK MCKINLEY, AND GEORGE M. DECHERD, JR.*

Atrioventricular nodal rhythm was first produced experimentally in 1903 by Engelmann, who assumed that the pacemaker of the heart was on the ventricular side of the A-V ring after exclusion of the S-A node by the first Stannius ligature. The following year, Lohmann supported these conclusions by work on the warm-blooded heart. In 1907, Sir James Mackenzie used the term "nodal rhythm" to designate a clinical arrhythmia which his polygraphic tracings show to have been what is now referred to as auricular fibrillation. Modern clinical usage of the term dates back to Belski, who in 1909 recorded five cases of A-V nodal rhythm.

Subsequent use of the electrocardiograph has facilitated the diagnosis of A-V nodal rhythm, although universal agreement on criteria has not yet been reached. In general, there are two large groups of cases in which there has been thought to be displacement of the cardiac pacemaker from the S-A node to the A-V node. In the first of these groups, the P wave, if visible, is inverted in Leads II and III, while upright in Lead I, indicating that the electrical axis of the atrium points up and to the left, in contrast to the normal direction down and to the left. In this large group there have been recognized three subdivisions: with the P wave before the QRS-complex, merged with it, or following it. The view has been held that these three possible relationships between the atrial and ventricular complexes indicate the location of the A-V nodal pacemaker in the upper, middle, or lower portions of the node (Ganter and Zahn, 1912). Scherf (1931), and more recently Katz and his colleagues (1944), have taken the position that

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the position of the P wave in relation to the QRS-complex means merely that there may be varying retrograde or antegrade conduction to the atrium or to the ventricle, and that their relative rapidity determines the relative position of the P and QRS-complexes (Fig. 1).

The second large group that has been thought to represent A-V nodal rhythm includes those cases in which the PR interval is 0.12 second or less, with upright P waves. The basis for this belief is the opinion, such as that expressed by Scherf and Schonbrunner (1935) and by Katz (1941) that at least

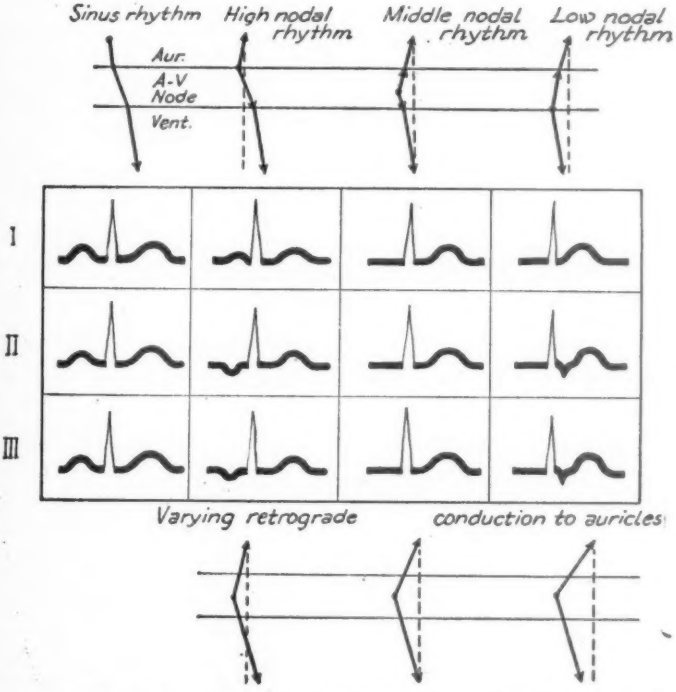

Fig. 1. Schematic representation of the three varieties of A–V nodal rhythm: above, with three postulated positions of the pacemaker in the node; below, with relative variation in retrograde and antegrade conduction.

0.12 sec. must be consumed in the passage of the excitation wave through the A-V junctional tissues. Hence, whenever the PR interval is equal to, or less than, that figure, it has been assumed that the pacemaker must actually be within these tissues themselves. Katz (1944) places the pacemaker in such instances in the A-V nodal tissues just to the right of the coronary sinus (Fig. 2), and includes these cases under the term "coronary" nodal rhythm. Recent studies of large groups of healthy young men (Stewart and Manning, 1944;

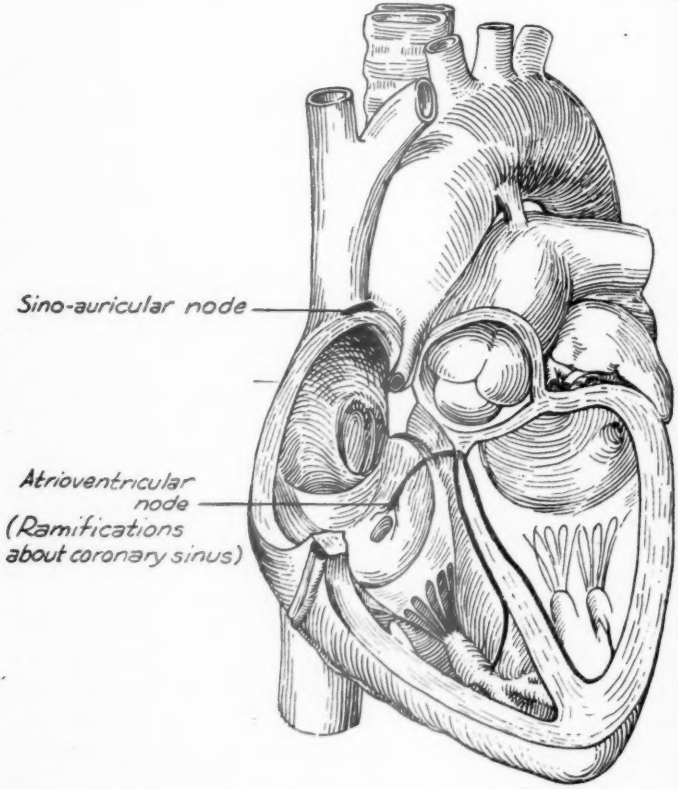

Fig. 2. Drawing of the heart, viewed from in front, with the right atrium cut away to show position of the S–A and A–V nodes.

Graybiel et al., 1944) such as make up air corps personnel, have shown that measurements of the PR interval show a reasonably symmetrical frequency distribution, and that it may be expected that in a small percentage of normal individuals the PR interval will be less than 0.12 sec. Also, our own (1944) studies of the direction and contour of the curves derived from the consecutive atrial electrical axes, have led us to the conclusion that the so-called "coronary" nodal rhythm is in fact merely S-A nodal rhythm, with conduction to the ventricles more rapid than usual.

It has been claimed (Rothberger and Scherf, 1927; Danielopolu and Proca, 1926) that aberrant intra-atrial conduction may lead to the production of negative P waves when the pacemaker is in the S-A node; and, conversely, that positive P waves may be seen when atrial excitation is from an A-V nodal pacemaker. This is based on animal experiments, using an ano-esophageal lead, and does not, we believe, have any yet proven applications to human electrocardiography.

Although A-V nodal rhythm is not extremely uncommon, it has been very little discussed in the cardiological literature. In 1941 Flaxman stated that he had been able to find descriptions of only fifty cases, to which he added twelve more. The purpose of this study was (1) a survey of the clinical features of patients whose electrocardiograms showed A-V nodal rhythm, and (2) a comparison of the clinical features of the various types of this mechanism disturbance. We have derived our material from the files of the Heart Station, which contain one or more electrocardiograms taken on ten thousand patients over a period of 13 years. A survey was made of the records of those patients whose electrocardiographic tracings satisfied the criteria cited above. Transient migration of the pacemaker to the A-V node, as for example, during carotid sinus stimulation, was not included, nor were those electrocardiograms showing a nodal pacemaker in the dying heart. The usual criteria for the presence of heart disease were employed; patients were considered to have some grade of heart failure if their functional capacity placed them in Class II, III, or IV of the New York Heart Association's

Criteria for Diagnosis of Diseases of the Heart. The electrocardiograms were considered abnormal, aside from the question of nodal rhythm, if there were the usually accepted abnormalities of the T waves or ST segments, or in conduction.

Results

There were 45 patients (0.45 per cent of the 10,000 patients included) with typical A-V nodal rhythm, i.e., with P waves that were negative in Leads II and III when they were visible. In 15 patients the P waves preceded the QRS, in 24 the atrial and ventricular complexes were merged, and in 3 cases the P waves followed the QRS-complexes; there were also 3 examples of A-V dissociation with interference. A PR interval of 0.12 second or less was found in 114 (1.14 per cent) patients. In 44 the interval was 0.10 sec., in 34 it was 0.11 sec., and in 36 it was 0.12 sec. The clinical data are summarized in Table I.

A-V nodal rhythm.

1. *With P waves preceding the QRS.* The PR interval varied from 0.12 to 0.22 sec., which supports the view that there may be variations in the relative speed of antegrade and retrograde conduction, even when the resultant places atrial before ventricular excitation. In 10 of the 15 cases definite heart disease was recognized; in 6 hypertension was the etiological agent, in 2 syphilitic aortitis, and in 2 others both hypertensive and syphilitic heart disease were present. Congestive heart failure of some grade was present in 7 patients; electrocardiographic abnormalities other than the nodal rhythm were seen in 11. Digitalis had been given to 5 patients, in one of whom there were evidences of overdosage.

2. *P waves in the QRS.* This group contained 24 cases, forming the majority of cases of nodal rhythm, as has been noted by others. Twenty (83 per cent) had heart disease, and in two-thirds of these an etiological factor was arterial hypertension; all showed electrocardiographic evidences of myocardial disease. Digitalis had been given to 11 patients, and in 5 it was thought that the dosage had been excessive.

3. In only 3 patients did the *P waves follow the QRS;* variable retrograde conduction was present, with the RP interval spontaneously as long as 0.36 sec., and longer under the influence of drugs (Decherd and Ruskin, 1943). All three patients were over 40 years of age, and each had arteriosclerotic heart disease, in one instance associated with arterial hypertension, and in two associated with syphilitic heart disease. Two were in congestive heart failure, and had received digitalis; one of these had signs of digitalis toxicity.

4. The 3 cases of *interference dissociation*, in which the atria responded to the sinus pacemaker, while the ventricles were controlled by a faster A-V nodal pacemaker, with intermittent A-V conduction, were also over 40 years of age; all had congestive heart failure. One had hypertensive heart disease, one syphilitic, and in the third these two etiological agents were both present. All had abnormal electrocardiograms, and all had received digitalis, one in excessive amounts.

Short P-R Interval ("coronary nodal rhythm"). In Table I are listed separately the clinical data for the cases with PR intervals of 0.10, 0.11, and 0.12 sec. While there are minor differences, it seems justifiable to group all the cases together, and to consider all with a short PR interval in one category. Slightly less than half of these patients were found to have heart disease; when heart disease was present, the etiology was at least in part hypertensive in 65 per cent of the cases. The data suggest that the shorter the PR interval, the lower the incidence of cardiac abnormalities. Heart failure and electrocardiographic abnormalities were seen in only one-fourth of the group; digitalis had been given to only 17 patients, and only one had received it to the point of toxicity. Heart disease was absent in slightly more than half of the patients; the largest group of patients without heart disease was that with psychiatric disturbances. Such disturbances were present in 28 cases (25 per cent of the group), including psychoneurosis either in the form of anxiety or hysteria, in 10 patients, a reactive depression in 10 patients, a true psychoses in 8 patients (4 schizophrenia, 3 manic-depressive, and 1 of undetermined type). Among this group were other diseases which

might be expected to contribute to imbalance of the vegetative nervous system, e.g., thyrotoxicosis, general paresis, pregnancy, bronchial asthma.

A *comparison* of certain clinical features of these two large groups, i.e., true A-V nodal rhythm, and short PR interval, reveals several pertinent facts (cf. Fig. 3). There is apparently no significant difference in the age or sex distribution. However, in the group with A-V nodal rhythm, heart disease was present in 80 per cent of the cases, while in only 48 per cent of those with short PR interval was this true.

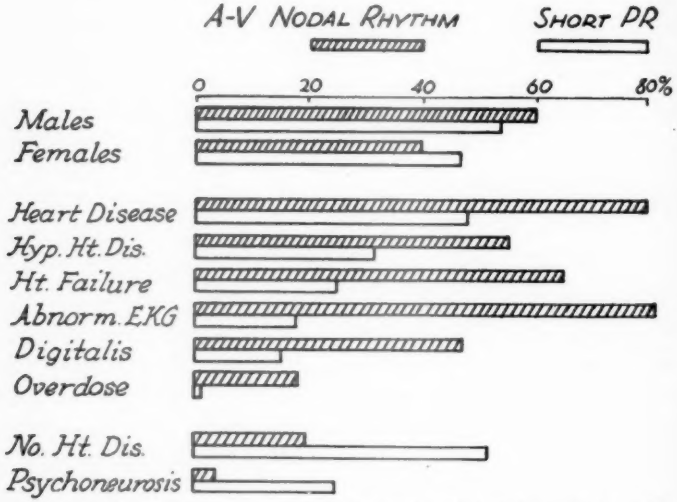

Fig. 3. Diagrammatic comparison of certain clinical features in A-V nodal rhythm and short PR interval.

The distribution of various etiological types of heart disease was virtually identical in the two groups, and hence is of no significance. In fact, this distribution agrees well with the incidence of these etiological types in our clinical material (Herrmann, Decherd, and Ruskin, 1942), and resembles that found by Flaxman (1941). However, when heart disease was present, it was not only present more often, but it was more

severe in the patients with true A-V nodal rhythm, as evidenced by the increased incidence, both relative and absolute, of other electrocardiographic changes, of cardiac failure, and of the necessity for digitalis administration.

A normal heart was far more common (52 per cent) among the group with a short PR interval than was the case among the patients with true nodal rhythm (20 per cent). Among the 59 cases with the short PR interval and without heart disease, the commonest abnormality was a psychiatric one. In contrast, psychiatric disturbances were present in only 2 patients with true A-V nodal rhythm; both had heart disease, one an anxiety state with hypertensive heart disease, and the second a psychosis with cerebral arteriosclerosis and congestive heart failure due to arteriosclerotic heart disease.

Comment

The ventricle responds to a pacemaker in the A-V node when (1) the normal S-A nodal pacemaker is depressed, (2) when A-V nodal rhythmicity is heightened, or (3) when both of these factors are active. In the experimental animal the activity of the S-A node may be inhibited by excluding it with a ligature (Engelmann, 1903), cooling (Ganter and Zahn, 1912), direct injury (Hering, 1910), or resection (Scherf, 1931). Similarly direct means may be employed for stimulation of the A-V node (Lohmann, 1904; Ganter and Zahn, 1912; Scherf, 1931, 1944).

Paradoxical results have been obtained from studies of nervous and drug influences, both in animals and in humans. S-A nodal depression by vagal stimulation, with resultant A-V nodal beats, is too well known to require comment; Hering (1905) produced nodal rhythm by stimulation of the left accelerans nerve, and Rothberger and Winterberg (1910) got the same result by simultaneous stimulation of the right vagus and the left accelerans. Gallavardin (1914) produced transient A-V nodal rhythm by ocular pressure, and found this effect was facilitated by administration of atropine. Wilson (1915) found that deep respiration caused a shift of the pacemaker to the A-V node. He likewise got transient A-V

nodal rhythm as an early effect following the injection of atropine, and Miller (1944) has reported a similar experience; this remarkable action has been ascribed to release of vagal inhibition of the A-V node before this effect is apparent at the S-A node. White (1915) observed that digitalis caused a change of cardiac mechanism from atrial flutter to fibrillation, and thence to A-V nodal rhythm; he also (1916) produced S-A nodal depression with ventricular escape beats, and interference dissociation (as it was later designated by Mobitz in 1923) by the employment of pressure over the carotid sinus, digitalis, and atropine. The amphotropic action of the carotid sinus reflex was emphasized by Weiss and Baker (1933); i.e., carotid sinus pressure might either cause A-V nodal rhythm, or if it was already present, convert it to sinus rhythm. These variable vagal effects were abolished by both epinephrine and atropine.

Clinically, Gallavardin and Gravier (1921) have subdivided atrioventricular rhythm into "rhythme nodale provoqué," due to nerve and drug influences; "rythme nodale transitoire" due usually to acute infections and rarely to chronic myocardial disease; and "rythme nodale permanente" with organic lesions producing total S-A block by destruction of the S-A node or its inferior atrial connections.

Miller (1944) has recently reviewed cases due variously to changes in vagal tone, infections, atropine, digitalis, and other drugs, and reported one instance in which A-V nodal rhythm followed exercise; he pointed out that either reflex vagal stimulation, or exercise, or atropine might convert A-V rhythm to normal sinus rhythm. Katz (1941) states that quinidine may cause A-V nodal rhythm, and Resnik and Lathrop (1925) have noted it during the apneic phase of Cheyne-Stokes respiration. Various anesthetics, including ether (Heard and Strauss, 1918), chloroform, ether, and cyclopropane (Kurtz et al., 1936; Meek et al., 1937) cause nodal rhythm in an astonishingly high percentage, 20–50 per cent, of cases that have been studied electrocardiographically during anesthesia. This is also true of the experimental animal (Allen et al., 1942).

Nervous reflexes and drug effects cause a transient nodal rhythm; a more persistent form has often been ascribed to disease of the heart muscle. Coronary thrombosis, with occlusion of the branch of the right coronary artery which supplied the S-A node, was found in one patient with A-V nodal rhythm (Geraudel, 1926); four of Flaxman's twelve cases (1941) had had coronary thrombosis, and many of Cowan's cases (1939) with interference dissociation had myocardial fibrosis. Jones and White (1927) likewise reported one case of A-V nodal and reciprocal rhythm resulting from coronary occlusion, and in a clinical study of acute coronary artery thrombosis, Master et al. (1937) found A-V nodal rhythm in 1 per cent of the group. Belski (1909) reported polygraphically recorded cases in patients with rheumatic fever, typhoid fever, and scarlet fever; Cowan et al. (1912) described three cases with acute ulcerative endocarditis involving the A-V node; and Hume (1913) found A-V nodal rhythm in patients with diphtheria, in one of whom inflammatory changes were found in sections of the S-A node. Wedd and Lichty (1941) found an association of nodal rhythm with rheumatic carditis, and Oettinger and Neslin (1932) noted this arrhythmia in as many as 7 per cent of patients with active rheumatic heart disease. The latter cases were in part of functional pathogenesis, since in many, normal sinus rhythm was restored by exercise or atropine. Both Oettinger and Neslin, and Cutts (1937) found interference dissociation relatively common in acute rheumatic carditis; other myocardial disease, particularly when digitalis has been given, is responsible for many cases (Zeisler, 1932).

We have found A-V nodal rhythm in 0.45 per cent of the patients on whom we have taken electrocardiograms; this agrees well with the figure of 0.43 per cent noted by Katz and Kaplan (1938). In agreement with Scherf and Boyd (1940), our data indicate that the majority of these cases are of the variety in which the P and QRS complexes are merged; it is rare to find the P waves following the QRS complex (cf. Table I). We cannot make a definite statement concerning the duration of this arrhythmia. Williams and James (1913) reported one case of 14 months' duration, as did Jones and

White (1927). One of our patients, an elderly woman with hypertensive heart disease, has shown A-V nodal rhythm, with inverted P waves in Leads II and III, preceding the QRS, in all electrocardiograms taken since 1939, a period of five years. The single tracing taken in 1933 showed migration of the pacemaker from the S-A to the A-V node. Our impression is that digitalis is most likely to produce the so-called "middle" type of nodal rhythm. This arrhythmia occurs chiefly in patients with heart disease, and the prognosis is determined by the nature and extent of the cardiac involvement, rather than by the presence of the nodal rhythm.

We feel that the finding of a short PR interval of 0.10–.12 sec. in 1.14 per cent of patients is merely evidence of more rapid than usual transmission of the excitation wave from the S-A node to the ventricle. Patients who have such electrocardiograms have heart disease in less than 50 per cent of the cases, and when the heart is abnormal, the severity of the disease is not as great as is found in patients with true A-V nodal rhythm. Since psychoneuroses, and other psychiatric disturbances, make up 25 per cent of this group, we suspect that nervous influences, perhaps sympathicotonia, may be responsible for the shorter PR interval. Stewart and Manning (1944) and Graybiel et al. (1944) obtained PR intervals as short as 0.09 sec. in a few of their healthy young aviators. For these reasons, and because of our previously cited studies, we feel that the term "coronary nodal rhythm" is a misnomer. Scherf's recent (1944) use of the term "coronary sinus rhythm" is unrelated, though potentially confusing.

Scherf (1940) has also studied the incidence of short PR intervals, finding it in 1.44 per cent of patients. He attributed it to quickened A-V conduction, and noted its association with hypertension, and less commonly, with rheumatic fever and beri-beri. We agree with his general conclusions, but have not noted any increased incidence of hypertension in this group as compared with our general population of cardiac patients.

Tabulation Comparing the Clinical Data in the Varieties of A-V Nodal Rhythm, and in the Presence of a PR Interval of 0.10–.12 sec.

Short P-R Interval

Tabulation Comparing the Clinical Data in the Varieties of A–V Nodal Rhythm, and in the Presence of a PR Interval of 0.10–.12 sec.

	A–V Nodal Rhythm						*Short P–R Interval*				
Position of P waves	Before QRS	In QRS	After QRS	Interference dissociation	Totals No.	Totals Per Cent	0.10	0.11	0.12	Totals No.	Totals Per Cent
Number of cases	15	24	3	3	45		44	34	36	114	
Age: range	10–90	10–80	40–60	40–60			10–90	10–70	5–95		
mode	50–60	60–70	50–60	40–50			50–60	30–40	50–60		
Males	7	15	2	3	27	60	25	15	21	61	53.5
Females	8	9	1	—	18	40	19	19	15	53	46.5
Heart Disease	10	20	3	3	36	80	17	15	23	55	48
Hypertensive	6	7	—	1	14	31	7	8	8	23	20
Hypertensive and arteriosclerotic	2	6	1	—	9	20	5	2	6	13	11.4
Hypertensive and syphilitic	—	1	—	1	2	4.5	—	—	—		
Arteriosclerotic	—	1	—	—	1	2.2	5	2	5	12	10.5
(myocardial infarct)	—	(3)	(1)	—	(4	9)	—	(2)	(2)	(4	3.5)
Myocardial and syphilitic	—	2	2	—	4	9	—	—	—		
Syphilitic	2	1	—	1	4	9	—	1	2	3	2.6
Rheumatic	—	2	—	—	2	4.5	—	1	2	3	2.6
Bacterial endocarditis	—	—	—	—			—	1	—	1	0.9
Heart Failure—											
Present	7	17	2	3	29	65	8	4	16	28	25
Absent	8	7	1	—	16	35	36	30	20	86	75
Electrocardiogram—											
Abnormal	11	20	3	3	37	82	10	8	8	26	23
Normal	4	4	—	—	8	18	34	26	28	88	77
Digitalis given	5	11	2	3	21	47	5	—	12	17	15
(overdosage)	(1)	(5)	(1)	(1)	(8	18)	(1)	—	—	(1	0.9)
not given	10	13	1	—	24	53	39	34	24	97	85
Quinidine	—	1	—	—	1	2.2	2	2	—	4	3.5
No Heart Disease	5	4	—	—	9	20	27	19	13	59	52
Psychiatric disturbance	—	—	—	—			11	14	3	28	25
Thyrotoxicosis	—	—	—	—			1	2	—	3	2.6
Pregnancy	—	—	—	—			2	—	—	2	1.7
General paresis	—	—	—	—			2	—	1	3	2.6
Acute nephritis	—	1	—	—	1	2.2	1	—	—	1	0.9
Bronchial asthma	—	1	—	—	1	2.2	1	—	—	1	0.9
Diabetic coma	—	—	—	—			1	—	—	1	0.9
Miscellaneous	5	2	—	—	7	14.7	8	3	9	20	17.5

SUMMARY

1. Electrocardiograms showing A-V nodal rhythm are obtained from patients having a high incidence of serious heart disease, as evidenced by the frequency of congestive heart failure, other electrocardiographic abnormalities, and the necessity of digitalis administration.

2. Electrocardiograms in which the PR interval is 0.10–.12 sec. are obtained from patients having a much lower incidence of heart disease, which, when present, is usually less severe. There is a striking incidence of psychiatric and other disturbances which may influence the vegetative nervous system.

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A RAPID FIXATION TECHNIQUE FOR TREMATODA AND HIRUDINEA

JOY BARNES CROSS*

With the method described in this paper an average of ninety well flattened liver flukes may be prepared in an hour, which is a fifteen-fold increase over the output when the usual procedure of tying the animals between glass slides is followed (Guyer, 1931, 1937; Galigher, 1934). Rapidity is particularly important because it is essential that only live specimens be utilized to avoid postmortem changes, and the period of survival after their hosts death is relatively short. Greater speed in preparation also makes it possible to take advantage of the large numbers of flukes which are obtainable only sporadically in some localities (i.e., a brief season about once in ten years near San Francisco).

Beef livers are kept at body temperature in thermos jugs during their transportation from the packing plant to the laboratory. Following the suggestion of Dr. J. L. Mohr, the livers are transferred to gallon glass jars, covered with mammalian Ringer's solution and stored over night in the refrigerator. During this time almost all of the flukes migrate into the fluid, and fewer specimens are damaged than if they are extracted by hand. The heavily discolored fluid containing the flukes is then poured into dissecting pans and the animals are separated from the debris by successive washings with the Ringer's solution. Animals treated in this way survive for a period of about sixteen hours after their removal from the refrigerator.

If flukes are tied between glass slides in the customary manner but are put into fixing solution in a black-bottomed pan, it may be seen that the pellicle adheres so tightly to the glass that the fluid can only contact a narrow peripheral border of the animal's body (Fig. 2). If cigarette paper is inserted between the fluke and the glass, complete penetra-

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tion is almost instantaneous. Fixation is readily ascertained by the change in the appearance of the animal from a transparent flesh color to an opaque bone-white that contrasts sharply with the dark bottom of the pan.

The following procedure is the result of these observations. A dark-bottomed dissecting pan is tilted in the manner shown in figure 1 and Susa's fixative is placed in the resulting trough

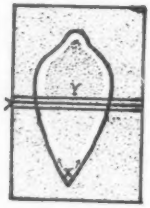

Figure 2

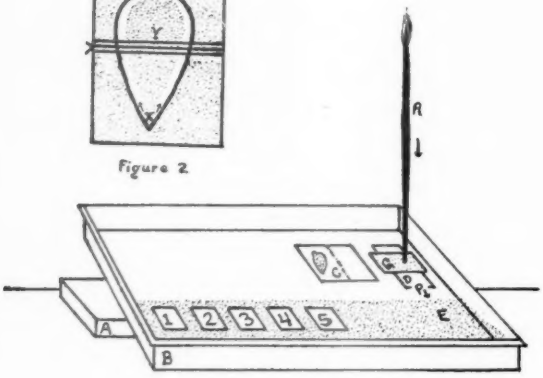

Figure 1

J. Cross

Fig. 1. A—Block for tilting pan: B—Dark-bottomed dissecting pan (9" x12"): C—Strip of toilet paper (" x 3"): D—Folded paper: E—Region occupied by fixing fluid: G—Piece of glass slide: P—Point where forceps grip paper: R—Brush, pressed downward by left hand: 1, 2, 3, 4, 5—Serial positions of flukes in fixing solution.

Fig. 2. Fluke tied between glass slides (usual method): X—opaque area affected by fixative: Y—Unaffected, transparent region.

(E), leaving approximately the upper two-thirds of the pan dry. A strip of toilet paper (C) is placed in the dry portion of the pan and a fluke is laid on one end and brushed flat with a camel's hairbrush. The opposite end of the paper is folded

over the animal and after the application of a few brush strokes a piece of glass (G) is laid on top in such a way that sufficient paper is left exposed (D) to afford a firm hold with paraffin-tipped forceps at the point P. Simultaneously, the left hand presses the brush handle (R) directly downward against the glass and the right hand manipulates the forceps and pulls the preparation forward and down the slope of the pan by "sled action" into the fixing solution.

The weight of the glass is sufficient to maintain the flattened state, and other flukes are prepared immediately in the same manner and are slid into adjacent positions (1, 2, 3, 4, 5) along the lower edge of the pan. By this time the first one has been in the solution long enough to become opaque and to retain its flattened condition. The glass and surrounding paper are removed and the fluke is transferred to a larger container of fixing fluid. The remaining animals are removed in turn to the larger jar of Susa's solution where they are left approximately twelve hours. It is possible after a little practice to manipulate two or three flukes at once on the same piece of paper and under the same glass.

With this method fixation is achieved superficially in two or three minutes in contrast to the older procedure in which four hours elapse before the string that binds the glass against the fluke is loosened to permit the contact of the fixing solution with the central portion of the animal. This shortening of the time requirement is particularly advantageous in respect to the quality of the preparation, for it is a generally accepted fact that rapid fixation minimizes the production of artifacts.

Leeches can also be fixed satisfactorily by this method, although even when they are partially narcotized, greater agility is required to get them "under glass" and the pressure of the brush tip must be stronger and must be maintained longer. About 75 per cent of the leeches that are fixed in this manner are secured with the proboscis extended and whole mounts stain well. The method is undoubtedly applicable to the preparation of other Trematodes, and it is imagined that it might make the latter steps in the preparation

of mounts of proglottids of Cestodes easier because it would insure their fixation in a flattened state more certainly than the usual procedure of winding the worms around glass plates.

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OBSERVATIONS ON EXPERIMENTAL BULLIS FEVER IN MAN

ROBERT K. BLAIR AND MADERO N. BADER*

Bullis fever was first described by Woodland, McDowell, and Richards (1943) as a new disease entity observed among soldiers at Camp Bullis, Texas. From their observations of one thousand cases, the authors described the disease as being characterized by high fever variable in duration and type, by generalized lymphadenopathy, photophobia, and marked leukopenia in most cases. Headache with general malaise was a constant symptom. The mortality rate was practically nil; only one death occurred among a thousand patients, and this was under extenuating circumstances.

"This very interesting outbreak is of more than local importance: It serves as a warning that when bodies of troops are engaged in operations in areas infested by ticks or mites, outbreaks of rickettsial fevers transmitted by these arthropods may constitute a serious war risk. Hithereto these fevers, being transmitted only from lower animals to man, and not from man to man, have nearly always occurred as sporadic cases. But the war has introduced a new set of conditions in which large outbreaks can be expected. In the whole of the Pacific area, and in Southeastern Asia, numerous cases of "scrub fever" (mite-borne typhus) are said to have occurred, sometimes in the form of considerable outbreaks. The fatality rates are said to have been low in most of the outbreaks, but they may be sometimes as high as 10 per cent, and even when they are low the disease is likely to be of serious military importance.

"The example of Bullis fever shows that the same kind of thing can happen in tick-infested areas, of which there

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are many in India, Africa, and America." (John Megaw, 1944.)

In addition to the mass outbreak of the disease among regular Army troops, similar cases also occurred among officers and men of the Texas State Guard stationed at Camp Bullis in summer, 1943. One thousand Guardsmen were divided into four groups, each of which was 250 men; they spent one week during the month of July in a tick-infested area where they were exposed to tick bites for several hours a day. A questionnaire was prepared by Major P. Mock, M.C., and Captain L. Anigstein, M.C., 49th Battalion, Texas State Guard, and sent through the Adjutant General's Office to the 1,000 men who had attended this camp. Of the 506 replies received, 10 gave a history of possible Bullis fever, two of which are listed here:

1. Did you become ill after returning fom Camp Bullis in July, 1943? *Yes.*
2. If so, how many days after leaving the Camp did you fall ill? *10.*
3. Did you develop any of the following signs?
   a. Chills: *Yes.*
   b. Fever; if any, for how many days? *Two days.*
   c. Headache: *Yes.*
   d. Skin rash: *No.*
   e. Swelling of lymph glands (kernels) of the groin, armpits or both: *Swelling of glands in neck and throat.*
   f. Your doctor's diagnosis, if any: *None.*
4. Your name, rank, and address: /s/ Sgt. W. G. T., Mission, Texas.

1. Did you become ill after returning from Camp Bullis in July, 1943? *Yes.*
2. If so, how many days after leaving the Camp did you fall ill? *14.*
3. Did you develop any of the following signs?
   a. Chills: *Yes.*
   b. Fever; if any, for how many days? *One week.*
   c. Headache: *Yes.*
   d. Skin rash: *Yes—sides of chest—type maculo papular eruption, two to three weeks.*

e. Swelling of lymph glands (kernels) of the groin, armpits, or both: *Both.*
f. Your doctor's diagnosis, if any: *None.*

4. Your name, rank, and address: /s/ 1st Lieut. L. B., Dallas 6, Texas.

In all cases a tick-bite history could be traced. Other positive cases in this group showed very similar reactions as found later in human volunteers as described in this paper.

As previously reported, a rickettsia was recovered from human Bullis fever patients which did not correspond to any rickettsial diseases available for study in this country. Livesay and Pollard, 1943.) Parallel investigations of ticks *(Amblyomma americanum)* collected at Camp Bullis yielded a rickettsial strain which proved identical with the human strain. (Anigstein and Bader, 1943.) These two strains were used in experiments on human volunteers.

The tick strain (BT) had been carried in guinea pigs through a number of passages; the 16th passage was used. The spleen of this guinea pig was ground in approximately 10 cc. of sterile skimmed milk, and frozen down in carbon dioxide ice. Before freezing, a sample of this material was taken and tests for sterility were run on chocolate agar and fluid thioglycollate media.

The first volunteers were inoculated with this material (to be known as the BT strain; viz., Bullis tick) on January 13, 1944. Four volunteers were used in the first series, and each received 2 cc. of material subcutaneously. On February 17, 1944, two more volunteers were used—one received the BT strain and the other the human strain (to be known as the BH strain; viz., Bullis human). The material for the BH strain was prepared in the same manner as that of the BT strain. On April 6, 1944, another pair of subjects were used; one received the BT strain and the other received the BH strain. The inoculum used on the four subjects of this group (April 6, 1944) was of lyophilized material. This was prepared by grinding the spleen of an infected guinea pig in

sterile skimmed milk, and drying the material over CO_2 ice. The vacuum in the tubes with the lyopholized material was replaced by nitrogen and sealed. Sterility tests were run on all material before it was lyophilized. Resuspension of the inoculum was made with saline.

Bullis fever in guinea pigs was found to be a very mild disease; in fact, only about 50 per cent of the animals react to the inoculation of the virus, while in some series the infection was inapparent, the guinea pigs remaining afebrile during three weeks of observation. (Bader and Anigstein, 1944.) The animals that did have fever showed no other clinical evidence of the disease except that occasionally there was a loss of weight. The mildness of the disease in experimental guinea pigs, as well as the relative mildness of the disease in nature, justified our belief that experimental production of the disease in humans would be safe. Actually, the human experimental cases, although broadly similar to the original disease clinically, were even milder than the average case observed in cases from Camp Bullis. All of the subjects used in these experiments were healthy, muscular, physically fit, young men who were free of fever and feeling perfectly well at the time of inoculation. The significant findings in each case are set forth in the table of case reports on page 105.

Discussion. One subject (No. 5) out of the nine failed to show any positive response following the inoculation, neither fever, malaise, nor leucopenia. Seven of the nine showed leucopenia. In each case in which differential counts were recorded the leucopenia was found to be at the expense of the polymorphonuclear leucocytes, producing a relative lymphocytosis. In two of these seven, the drop in white cell count was the only positive finding, the experimental disease being so mild as not to produce fever, headache, or any feeling of illness in the subject. In two of the nine subjects the clinical syndrome was fairly typical and moderately severe: One of these (No. 8) a husky white male, 35 years old, developed fever ranging between 99 and 100° for the first six days, then dropping to normal for another six days and finally rising again for the last five days of the disease. Dur-

Case No.	Day of onset of fever	Duration of fever in days	WBC	Polys %	Source of Material	Clinical Symptoms
1	9	4	8,200		BT	No complaints except fever (99 to 99.2 ea. afternoon).
			8,250			
2	—	—	13,350		BH	No fever. Occipital headache for 4 days.
			8,650			
3	—	—	9,450		BT	No fever, no complaints (leukopenia only).
			4,900			
4	7	5	10,200			No complaints except fever
			3,700		BH	(99 to 99.4 ea. afternoon).
			4,950			
5	—	—	7,950	48	BH	No fever, no complaints; no leukopenia.
			12,850	55		
6	10	16	6,900	45		Low-grade fever, lymphadenopathy, severe headache, malaise, nausea, weight loss. No respiratory symptoms.
			7,000	52	BT	
			4,400	36		
7	11	19	8,500	64		Low-grade fever, slight headache.
			7,800	80		
			9,000	48	BT	
			4,600	48		
			9,700	50		
			9,500	40		
8	13	28	6,600	64		Low-grade fever, prolonged general malaise, weight loss, headaches, nausea.
			7,400	66		
			5,800	64		
			6,000	44	BH	No respiratory symptoms.
			6,200	28		
			4,500	30		
			7,300	28		
9	9	24	11,100	60		Low-grade fever, moderate general malaise, persistent slight headache, occasional nausea.
			8,000	30		
			7,800	44	BT	
			8,700	46		
			6,100			

ing this time he complained of constant frontal and occipital headaches; after the first ten days, the symptom of nausea appeared. There was also photophobia, burning of the eyes, and rather severe general malaise associated with the fever. The patient lost 12 pounds weight and felt weak for four weeks, after which time he began to improve in weight and strength, going on to complete recovery. The other (No. 6), a tall, muscular, white man, 28 years old, began to run fever after ten days of incubation, showing an intermittent type and ranging between 99 and 100°. After the first fever attack, swelling of axillary and inguinal lymph nodes developed; the

glands became larger with the next two days, and generalized lymphadenopathy lasted for the ten days' fever period, along with pronounced frontal headache, general malaise, nausea, aching, and weight loss of 9 pounds. The lymphadenopathy and other symptoms gradually disappeared as the fever subsided, with recovery of the patient approximately sixteen days after onset. None of the cases showed any signs or symptoms referable to the respiratory tract, which would distinguish the milder cases from the ordinary mild case of influenza. The aching was not of the severe, prostrating type associated with dengue fever, and the general clinical course of the cases did not conform to that of dengue. There were no frank chills, and the blood smears were always negative for plasmodia malariae. In short, this experimentally produced syndrome resembled no other disease so much as that described by Woodland and associates and called "Bullis fever."

Conclusions. Two strains of an infectious agent recovered from human patients and from ticks of the Camp Bullis area were used for human experiments. Both strains had been maintained in guinea pig passage for a number of generations. Fresh or lyophiled material was used for the inoculum. All of the patients save one inoculated with these strains showed the syndrome of Bullis fever as described by Woodland and associates; however, most were of a mild nature. Patients inoculated with material isolated from ticks *(A. americanum)* showed the same reaction as those inoculated with the BH strain. These results offer further evidence that the rickettsial isolated from human patients (Livesay and Pollard, 1943) and from ticks (Anigstein and Bader, 1943) is specific for the disease diagnosed as Bullis fever.

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FIELD INVESTIGATIONS PERTINENT TO BULLIS FEVER

Preliminary Report on the Species of Ticks and Vertebrates Occurring at Camp Bullis, Texas*

James M. Brennan†

The name "Bullis fever" has been used to designate an apparent new disease entity (1) presumed to be rickettsial, (2) which is endemic at Camp Bullis, Texas. This disease is thought to be arthropod-transmitted (3) the lone-star tick, *Amblyomma americanum*, being strongly suspected to be the vector (4, 5) although it is recognized that there is a possibility that other parasites, particularly the chigger, *Eutrombicula alfreddugesi*, may be concerned. Because it appears so probable that this disease has its source in nature, a study of the arthropod and vertebrate faunas was undertaken during the year beginning June, 1943. This study was made particularly with the object of obtaining data pertaining to the occurrence, distribution, and host relationships of ticks. The present paper is limited to a brief statement concerning each species of tick, and to lists of the vertebrates found on

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And, finally, to the many officers and enlisted men of the Army, who, through their interest, contributed materially to the work, much credit is due. This report was submitted for publication on January 13, 1945.

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the area. The complete data for the tick, *Amblyomma americanum*, will be embodied in a later report.

Camp Bullis, a sub-post of Fort Sam Houston, used for training and maneuvers, is located in the hills of Bexar and Comal counties, about 18 miles north of San Antonio, Texas. It has a mean elevation of approximately 1,200 feet and includes roughly 30,500 acres on which a federal game preserve is established. Abundant limestone outcroppings characterize the numerous small hills and ridges. Relatively small expanses of lowlands show evidence of once having been under cultivation and toward the northern end of the area abandoned pasture land is apparent. There is no varied plant association. The vegetation is dominated by junipers, oaks, thorny shrubs, tangles of vines and grasses. Semi-arid conditions prevail, the annual precipitation scarcely exceeding 22 inches. Except for the Cibolo River at the northern limits and the Salado Creek which traverses much of the reservation (both streams frequently dry) waterways and standing water are negligible. Many roads and innumerable interlacing trails pattern the entire area.

In the course of a survey conducted at Camp Bullis during the summer and early fall of 1943 and some additional field work in the spring and summer of 1944, the collecting of at least 700 specimens of mammals, birds, and reptiles supplemented by numerous observations afield enabled the author to compile tentative check lists of their species and the ticks indigenous to the region. Since the existing data on the faunal composition are inadequate or obsolete and since facilities were limited, it is recognized that the lists incorporated herein are incomplete, especially with respect to the avian population. Any data pertaining to the incidence and abundance of species were derived only by estimate through repeated observations, augmented to some extent by the actual number of specimens collected, hence such information is conveyed to the reader by purely relative phraseology under columns headed "occurrence" in the accompanying lists.

The following species of ticks were recorded:*

*Determinations by R. A. Cooley.

1. *Amblyomma americanum* (Linn.); from numerous hosts including man. Animals from which this species was recovered are shown by the letter A after the common name in the mammalian and avian lists. The letters a, n, and l indicate whether adult, nymphal or larval ticks were recorded. This recently established vector of spotted fever (6) is extremely abundant, is a source of great annoyance to man, and is a considerable factor in lowering the morale of troops on bivouac.

2. *Amblyomma inornatum* (Banks); from jack rabbit. Of numerous rabbits examined, only two records of one adult tick were obtained.

3. *Haemaphysalis ~~leporis~~-palustris* (Packard); adults and nymphs from jack rabbit and cotton tail rabbit, one crawling adult from a deer, nymphs only from bobwhite quail, adults and nymphs from cardinal, and nymphs and larvae from meadowlark. This tick, not known to attack man, but a vector of tularemia and spotted fever among animals, is fairly abundant. Except for the presence of *H. leporis-palustris* on 7 out of 12 meadowlarks taken in February, 1944, its occurrence on nearly 400 other bird specimens examined was negligible. Additional winter data conceivably might alter this picture.

4. *Ixodes texanus* (Banks); adults only from raccoon and cacomistle. Not abundant.

5. *Ixodes scapularis* Say ; adults from coyote, deer and man, nymphs and larvae from the lizards: *Leiolepisma unicolor*, *Sceloporus olivaceus*, and *S. undulatus fasciatus*. No records were obtained on adults of this species until late October. It was not taken abundantly nor frequently.

6. *Ixodes* sp. from a wren. One record of larvae.

7. *Dermacentor variabilis* (Say); one adult from a coyote. This disease-transmitting species attacks man readily. It is exceptionally rare and hence of no importance in the area.

8. *Dermacentor albipictus* (Packard); adults and nymphs from deer. The first records on this winter species were obtained late in October. It occurs commonly, but is not abundant.

9. *Otobius megnini* (Dugès); the spinose ear tick, nymphs and larvae from deer, taken infrequently.

10. *Ornithodoros talaje* (Guérin-Méneville); from the nests of cliff swallows. This tick readily attacks man, but is rare and has a restricted distribution.

11. *Ornithodoros* sp.; one record of larvae from nestling cliff swallows. May be *O. talaje.*

12. *Argas persicus* (Latreille); one record of a larva from black vulture. This tick is of rare occurrence.

There are no domestic animals at large on the reservation, the few that are present being confined to the immediate vicinity of a small number of quarters, hence these are not included in the following lists.

MAMMALIAN LIST*

Species	Common Name	Occurrence
Odocoileus virginianus	White-tailed deer (A) a. n. l.	Very abundant
Procyon lotor fuscipes	Raccoon (A) a. n. l.	Common
Bassariscus astutus flavus	Cacomistle, ring-tail (A) a, n.	Fairly common
Urocyon cinereoargenteus, subsp.	Gray fox (A) a. n. l.	Abundant
Canis latrans	Coyote.	Rare
Sciurus niger limitis	Fox squirrel (A) n. l.	Common
Mephitis mephitis mesomelas	Striped skunk (A) n. l.	Common
Conepatus mesoleucus mearnsi	Hog-nosed skunk (A) a. n. l.	Scarce
Didelphis virginianus	Opossum (A) n. l.	Fairly common
Sylvilagus floridanus chapmani	Cottontail rabbit (A) a. n. l.	Fairly common
Lepus californicus merriami	Jack rabbit (A) a. n. l.	Very abundant
Citellus mexicanus parvidens	Mexican ground squirrel (A) l.	Scarce, limited
Citellus variegatus buckleyi	Rock squirrel (A) n. l.	Scarce
Sigmodon hispidus texianus	Cotton rat (A) n. l.	Scarce
Rattus rattus alexandrinus	Roof rat (A) l.	Scarce, limited

*Determinations by the author, W. J. Hamilton, Jr., L. R. Dice, A. J. Kirn.

Dasypus novemcinctum texanum	Armadillo (A) n.	Very abundant
Lynx rufus, subsp.	Bobcat.	Reported, not verified
Tadarida mexicana	Mexican free-tailed bat.	Fairly common
Eptesicus fuscus, subsp.	Big brown bat.	Scarce
Peromyscus leucopus texanus	White-footed mouse.	Scarce
Peromyscus pectoralis laceianus	White-footed mouse.	Fairly common
Neotoma sp.	Wood rat.	Rare

The relative scarcity of small rodents (*Peromyscus*, *Neotoma*, *Sigmodon*, *Citellus*, etc.) is noteworthy. In this instance the findings of the author were substantiated by the observations of Hamilton (7), who states, "A thorough survey of supposedly suitable areas revealed no evidence of small rodents," and later adds, "The present scarcity of small rodents on this area might be attributable to a low in their population cycle, were it not for the absolute absence of old sign." It would appear then, that the role of small rodents as hosts for ticks at Camp Bullis is of negligible importance.

Among the mammals found to support moderate to heavy tick populations consistently are deer, rabbits, foxes, skunks, and raccoons. The deer, whose numbers have been estimated variously from 2,000 to 5,000, but according to Hamilton (*loc. cit.*), probably do not exceed 1,500, are the principal hosts for the adults of *Amblyomma americanum*.

AVIAN LIST*

Species	Common Name	Occurrence
Ardea herodias	‡Great blue heron.	Rare, limited
Ardea candidissima	‡Snowy heron.	Rare, limited
Butorides virescens	‡Green heron.	Rare
Querquedula discors	Blue-winged teal.	Rare, limited
Coragyps atratus	Black vulture.	Abundant
Cathartes aura	Turkey vulture (A) a, n.	Common
Accipiter velox	‡Sharp-shinned hawk.	Rare
Accipiter cooperi	‡Cooper's hawk.	Rare

*Determinations by the author, D. E. Davis, R. W. Quillin, A. J. Kirn, W. J. Hamilton, Jr.

AVIAN LIST—Continued*

Species	Common Name	Occurrence
Buteo swainsoni	Swainson's hawk (A) n. l.	Scarce
Buteo borealis	Red-tailed hawk (A) n. l.	Scarce
Circus hudsonius	†Marsh hawk.	Scarce
Falco sparverius	Sparrow hawk.	Scarce
Colinus virginianus	Bobwhite quail (A) a. n. l.	Abundant
Meleagris gallopavo	Wild turkey (A) n. l.	Common
Tringa solitaria	Solitary sandpiper.	Rare
Totanus melanoleucus	†Greater yellow legs.	Rare
Oryecus vociferus	†Killdeer.	Rare
Zenaidura macroura	Mourning dove (A) n. l.	Abundant
Columbigallina passerina	Ground dove (A) l.	Scarce
Geococcyx californianus	Road runner (A) n. l.	Scarce
Coccyzus americanus	Yellow-billed cuckoo (A) n. l.	Common
Otus asio	†Screech owl.	Scarce
Tyto alba	†Barn owl.	Rare
Bubo virginianus	†Western horned owl.	Rare
Chordeiles minor	Nighthawk.	Common
Phalaenoptilus nuttalli	Poor will (A) n. l.	Common
Antrostomus carolinensis	Chuck-wills-widow (A) l.	Scarce
Chaetura selagica	†Chimney swift.	Scarce
Archilochus alexandri	Black-chinned humming bird.	Fairly common
Megaceryle alcyon	†Belted kingfisher.	Scarce
Centurus aurifrons	†Golden-fronted woodpecker.	Common
Dryobates scalaris	Ladderback woodpecker.	Common
Muscivora forficata	Scissor-tailed flycatcher (A) l.	Fairly common
Myiarchus crinitus	Crested flycatcher (A) n.	Fairly common
Myiarchus cinerascens	Ash-throated flycatcher.	Scarce
Empidonax minimus	Least flycatcher (A) n.	Fairly common
Pyrocephalus rubinus	Vermilion flycatcher.	Fairly common

*Determinations by the author, D. E. Davis, R. W. Quillin, A. J. Kirn, W. J. Hamilton, Jr.

†These species not collected.

AVIAN LIST—Continued*

Species	Common Name	Occurrence
Myiochanes richardsonii	Western wood pewee (A) n.	Rare
Myiochanes virens	†Wood pewee.	Common
Sayornis phoebe	†Phoebe.	Scarce
Petrochelidon albifrons	Cliff swallow.	Common, limited
Baeolophus atricristatus	Sennett's titmouse (A) n. l.	Common
Baeolophus inornatus	Plain titmouse.	Common
Auriparus flaviceps	†Verdin.	Scarce
Penthestes carolinensis	Plumbeous chickadee.	Common
Thryomanes bewicki	Bewick's wren (A) n. l.	Common
Catherpes mexicanus	Canyon wren (A) n. l.	Scarce
Thryothorus ludovicianus	Carolina wren (A) n. l.	Fairly common
Heleodytes brunneicapillus	Cactus wren.	Rare
Mimus polyglottos	Mockingbird (A) n. l.	Abundant
Sialia sialis	†Bluebird.	Fairly common
Turdus migrat orius	†Robin.	Rare (occurs after Oct. 1)
Lanius ludovicianus	White-rumped shrike (A) n.	Rare
Vireo griseus	White-eyed vireo.	Common
Dendroica caerulescens	Black-throated blue warbler (A) l.	Rare
Dendroica aestiva	†Yellow warbler.	Rare
Dendroica chrysoparia	†Golden-cheeked warbler.	Fairly common
Mniotilta varia	†Black and white warbler.	Fairly common
Oporornis formosus	†Kentucky warbler.	Fairly common
Icteria virens	Yellow-breasted chat (A) n. l.	Scarce
Icterus spurius	Orchard oriole.	Scarce
Molothrus ater	Cowbird.	Common
Sturnella magna	Meadowlark.	Common after Oct. 1
Xanthocephalus xanthocephalus	Yellow-headed blackbird.	Rare
Piranga rubra	Summer tanager (A) n. l.	Common

*Determinations by the author, D. E. Davis, R. W. Quillin, A. J. Kirn, W. J. Hamilton, Jr.

†These species not collected.

Guiraca caerulea	Western blue grosbeak (A) l.	Scarce
Hedymeles melanocephala	†Black-headed grosbeak.	Rare
Carpodacus mexicanus	House finch (A) l.	Fairly common
Spinus psaltria	Arkansas goldfinch (A) n. l.	Common
Richmondena cardinalis	Gray-tailed cardinal (A).	Abundant
Pipilo sp.	†Towhee.	Rare
Passerina ciris	Painted bunting (A) n. l.	Common
Passer domesticus	English sparrow.	Common. limited
Chondestes grammacus	Western lark sparrow (A) n, l.	Abundant
Aimophila ruficeps	Rock sparrow (A) n. l.	Common
Aimophila cassini	Cassin's sparrow.	Fairly common
Pooecetes gramineus	Vesper sparrow.	Abundant after Oct. 1
Spizella pusilla	Field sparrow (A) a. n. l.	Common
Fringillidae	Sparrow (A) n, l.	

†These species not collected.

Of ecological significance is the scarcity of raptorial birds presumably attributable to the lack of small rodents. However, some increase in the numbers of hawks was observed in the fall.

The precocial birds invariably exhibited moderate to heavy tick infestation throughout the season. Among the altricial birds, the ground and thicket-dwelling species such as the wrens, cardinals, and certain sparrows consistently showed light to moderate tick infestation.

As a group, the birds are of no importance as hosts for adult ticks, but for the engorgement and dissemination of larval and nymphal ticks, the birds, because of their habits and great numbers, present a complex problem of menacing proportions.

The reptiles (snakes and lizards only) of the region are included because, as a group, they were observed to be the most important natural hosts for the human chigger, *Eutrombicula alfreddugesi*. This species of mite likewise was frequently observed on rabbits and several species of birds, but, with

the occasional exception of rabbits, never in the numbers noted on reptiles. It was not uncommon to take upwards from 60 individuals from the gular folds of certain lizards and more than a thousand were recorded from a chicken snake of average size.

In the accompanying list, the letter E following the common name designates the species from which chiggers were recorded.

REPTILIAN LIST*

Species	Common Name	Occurrence
Crotalus atrox	Diamond-back rattlesnake (E)	Common
Agkistrodon mokeson laticinctus	Copperhead snake	Scarce
Micrurus fulvius	Coral snake	Common
Thamnophis sauritus proximus	Western ribbon snake (E)	Common
Thamnophis marcianus	Marcy's garter snake (E)	Common
Thamnophis eques	White-bellied garter snake	Common
Masticophis flagellum testaceus	Coach whip snake (E)	Common
Masticophis taeniatus girardi	Striped racer (E)	Fairly common
Elaphe obsoleta confinnis	Chicken snake (E)	Common
Natrix rhombifera rhombifera	Diamond-back water snake	Fairly common
Heterodon contortrix contortrix	Hog-nosed snake (E)	Fairly common
Salvadora lineata	Patch-nosed snake	Scarce
Opheodrys aestivus	Rough green snake (E)	Common
Sonora taylori	Taylor's ground snake	Scarce
Tantilla gracilis	Graceful tantilla	Fairly common
Leptotyphlops dulcis dulcis	Worm snake	Fairly common
Gerrhonotus infernalis	Plated lizard	Scarce
Cnemidophorus gularis	Race-runner (E)	Abundant
Sceloporus olivaceus	Large-scaled swift (E)	Abundant
Sceloporus undulatus fasciatus	Small-scaled swift (E)	Fairly common
Sceloporus torquatus poinsettii	Poinsett's swift	Scarce
Leiolepisma unicolor	Brown skink	Abundant
Phrynosoma cornuta	Horned toad (E)	Rare
Crotaphytus collaris collaris	Collared lizard (E)	Common

*Determinations by H. Graham Netting and A. J. Kirn.

Summary

1. Twelve species of ticks occur at Camp Bullis. Of these, but one, *Amblyomma americanum*, because of its extreme abundance and because it readily attacks man, appears to be of any importance. It was recorded from 15 species of mammals and 33 species of birds.

2. Twenty-two species of mammals are listed from the area. The scarcity of small rodents is noteworthy. The deer is the most important host for the adults of *Amblyomma americanum*.

3. Seventy-nine species of birds are recorded from Camp Bullis. Of these, the ground-inhabiting and bush-dwelling species were consistently infested with immature stages of *Amblyomma americanum*. Because of their habits, the birds occupy an important ecological niche relative to the spread and maintenance of the tick population.

4. Sixteen species of snakes and eight species of lizards are listed. The reptiles as a group constitute the most important natural hosts for the chigger, *Eutrombicula alfreddugesi*.

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RETICULO-ENDOTHELIAL IMMUNE SERUM (R.E.I.S.): I. ITS ACTION ON SPLEEN *IN VITRO*•

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The second issue of the *American Review of Soviet Medicine* is notably devoted to the question of anti-reticular-cytotoxic serum (ACS) as it has been developed in the U.S.S.R. primarily under the leadership of Alexander Bogomolets. The reticulo-endothelial system is reappraised as the "physiological system of the connective tissues" and the importance of the technique for its control is discussed by Bogomolets.

The categories of activities which may be influenced by ACS are reported to include: (1) *Plastic action*—the stimulation of connective tissue cell growth; (2) *Protective function*—building resistance to infection by phagocytic action and antibody formation; and (3) *Trophic action*—promoting improved nutrition. Increased permeability of the "hemo-parenchymal barrier," dilation of capillaries, migration of monocytes, increase in the amount of complement, of the opsonic index and of phagocytic activity as a result of administration of ACS are given as demonstrable indicators of the mechanism of action. When used in minute dosage ACS is reported to exert a stimulating effect on the reticulo-endothelial system (RES). When the same serum is employed at large doses the effect is said to be "cytotoxic." We believe that the designation reticulo-endothelial immune serum (R.E.I.S.) might be more suitable. This name would describe the serum in more general and immunological terms without insistence on its "cytotoxic" properties.

As a result of a large amount of experimental work carried on by Soviet workers, Bogomolets reports (1943) that their ACS preparations have proven particularly valuable in the management of rickettsial diseases, in which the RES is known to be the tissue involved. It is also claimed that ACS

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is of therapeutic value in a wide variety of pathological conditions, including hypertension, tuberculosis, malignancy, and surgical infections. Moreover, it is said to aid wound healing and may prove of great merit in promoting longevity. Should these phenomena be correlated with the RES they would add a conspicuous weight to the already existent evidence of the importance of RES in health and disease (Baker, 1934). Studies in the course of various infections in experimental animals following "blockading" or splenectomy have demonstrated in many instances an apparent lack or reduction of antibody production (Jungeblut, 1928).

Recently Dougherty, Chase and White (1944) not only supported the view that the RES in general is concerned with the production of antibodies, but also presented evidence that the antibodies are concentrated chiefly within lymphocytes.

It has been also demonstrated by McMaster and Hudach (1935) that the formation of agglutinins takes place within draining lymph nodes. Similar observations on popliteal lymph nodes were made by Ehrich and Harris (1942). Recently these authors suggested (1945) that, while micro and macrophages react with antigenic substances, the final process of antibody formation may be the work of lymphocytes. Finally, an endocrine mechanism for the liberation of antibodies from lymphoid tissue is offered by Dougherty, White and Chase (1944) who report that adrenal cortical injections enhance agglutinin titers to sheep erythrocytes.

It has also been noted that the reticulo-endothelial system is affected by chemotherapy (Jaffe, 1931). The effects of such action can be demonstrated by the use of thorium dioxide blockade. This field has been extensively reviewed and enriched by Maher in a recent monograph (1944). Important to these observations is the report by Pinkerton (1944) that continuous and high dosaging of animals with sulfonamides produces an increased susceptibility to rickettsial infections—diseases characterized by damage of the RES. In contrast, para-amino-benzoic acid, an antagonist to sulfonamides (Woods, 1940), has recently proven of beneficial effect in the treatment of typhus fever (Yoemans, Snyder, Murray, Zarafonetis and Ecke, 1944). These promising results invite

further experimental study of the RES in relation to chemical agents which, in some instances, probably act via enzyme systems.

With the belief that the work of Soviet scientists is deserving of additional study with diverse methods, a group project was initiated in our laboratories for the study of the biological properties of R.E.I.S. and the potentialities of its application. *In vivo* and *in vitro* experiments designed to investigate both the inhibitory action of R.E.I.S. at strong concentrations and its possible stimulating action when sufficiently diluted are in progress.

This report is limited to observations made with tissue culture techniques on explants of embryonic and post-embryonic spleen fragments.*

Materials and Methods

The preparation of antisera for our experiments followed the methods used by the Soviet workers and described by Marchuk (1943). Details of serological procedures developed will be published in a separate report. Essentially all preparations involved the following steps.

One to two parts of spleen washed in saline were added to 8 or 9 parts of bone marrow and ground in a mortar with alundum. Saline in proportion to five times the weight of minced tissue was added. One-half of this mixture was centrifuged 3–4 minutes at 1,000 r.p.m. The supernatant, exclusive of a fatty layer, was injected intravenously while the uncentrifuged moiety was given by mouth. Rabbits were used in preparing antisera for the chick, rat, and guinea pig antigens while goats were used for anti-rabbit, cow, and human preparations.

The antigen was injected intravenously at four- or five-day intervals. Nine days after 4 to 6 such injections the experimental animals were bled and serum was collected in sterile

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vials. The following table represents the data concerning the antisera used in the present study along with titers established by complement-fixation tests.

R.E.I.S.	Number of intravenous and oral administrations	Complement fixation titer
Anti-chick	5	1–1200
Anti-rat	5	1–1600
Anti-guinea pig.....	4	1–1280

All experiments were carried out in hanging drop preparations and were incubated at 37.5° C. Spleen fragments were obtained from chicks incubated for 8–21 days, and from one- to four-day-old hatched chicks. Mammalian spleens were collected from embryos or from one- to four-day-old newborn animals. The culture medium consisted of heparinized rooster plasma obtained within five days of the experiment, and embryonic extract (in final concentration of 5–15 per cent) derived from chick embryos incubated from 6 to 9 days. R.E.I.S. undiluted or diluted with Tyrode's solution to produce any desired final concentrations was mixed with embryonic extract. Fragments were placed in a drop of this solution and an equal amount of plasma was mixed in to form a firm clot. Since cultures of spleen show liquefication of plasma clots by the third day of incubation, the explants were generally fixed before the 60th hour in order to preserve the pattern of the outgrowth.

In most experiments four preparations were made for each condition under study. For each experiment controls were prepared of both the serum of an untreated rabbit and of medium containing no mammalian serum but with the same concentration of embryonic extract as that used in the other cultures. Preparations to be stained with Giemsa were fixed in Regaud's fluid, absolute methyl alcohol or Dawson's fluid. Various staining procedures were tried for routine records, the most satisfactory of which was found to consist of fixation overnight in 10 per cent formalin in normal saline followed by staining overnight in 20 per cent Harris' hematoxylin.

Chlorozol black (Renol black G extra), which has been reported valuable as a vital dye (J. R. Baker), was added directly to the plasma used in some series of cultures.

Since the cultivation of animal tissues remains an empirical technique, expriments were usually designed to test the reactions of spleens of several animal species to various sera while other variables were kept constant. Consequently, specific cellular effects were adequately controlled. In reporting the data the Roman numerals which designate the results of each experiment are used.

For the purposes of this study living cultures were used and more than 900 of them were fixed, stained and examined. The varieties of patterns of cellular outgrowths influenced by R.E.I.S. were studied on several hundred photomicrographs.

Observations on Tissue Reactions to R.E.I.S.

In the course of our experiments several patterns of splenic outgrowth became evident, each of them characteristic for the particular effect of R.E.I.S.

When grown *in vitro* under normal conditions, cells from spleen fragments migrate radially in such a way as to produce very uniform circles (fig. 1). However, when such explants are exposed to various stimuli the outgrowth may become eccentric due to chemotaxis (Chambers and Grand, 1936, and Pomerat, in press).

In comparison with untreated controls, splenic explants may also produce symmetrical but exaggerated outgrowths under the influence of certain substances added to the medium. In this way a quantitative measurement of R.E.I.S. stimulation might be made. The inhibitory action of R.E.I.S. can be readily observed by a marked restriction of outgrowth as previously described by Pomerat and Anigstein (1944) and seen in Figures 3, 4, 5, and 6.

In addition to uniform cellular migration, splenic fragments may show differentiated zones of concentration of particular cell types.* It is well known, for example, that granu-

*Studies by Danchakoff (1916) and by Rioch (1923) were found valuable references for the cytology of embryonic chick spleen.

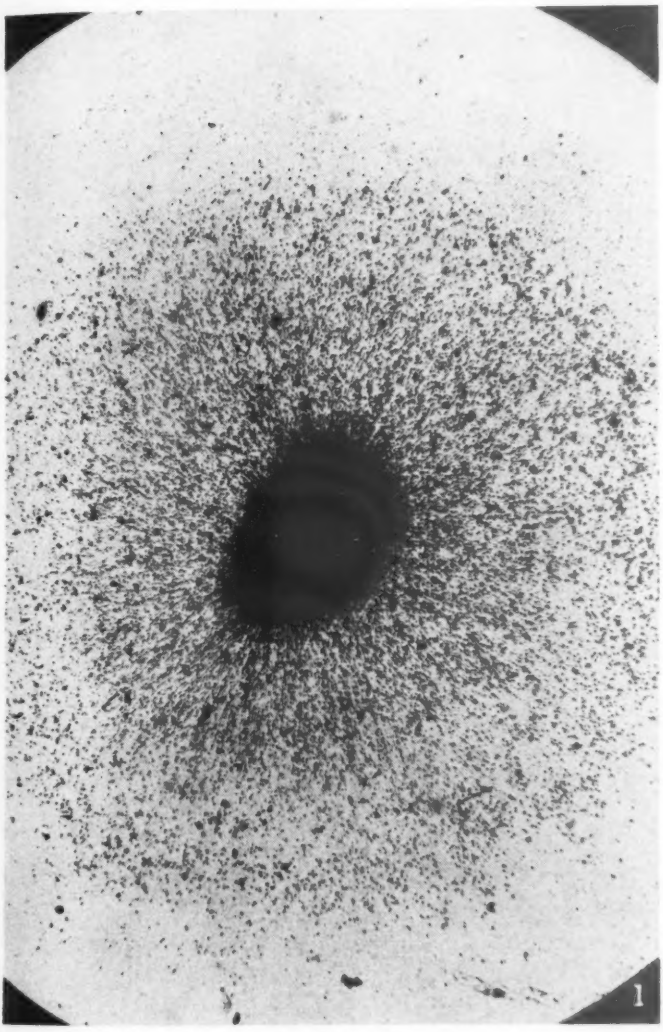

Fig. 1. Chick embryo—18 day. Spleen fragment cultivated for 48 hours *in vitro*. Control—28X.

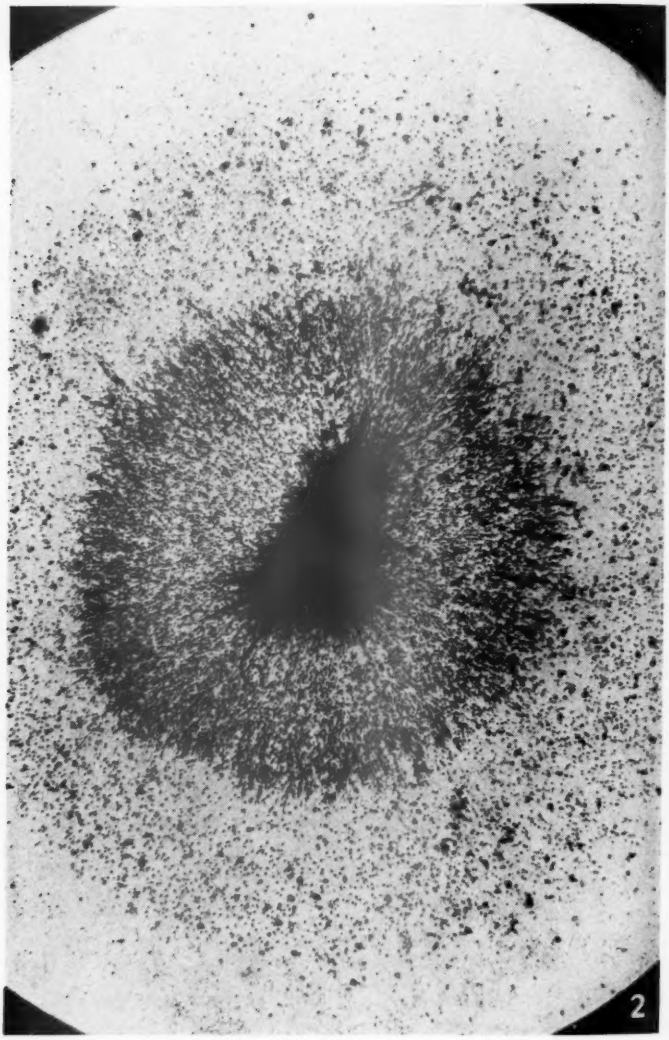

Fig. 2. Chick embryo—18 day. Spleen fragment cultivated for 48 hours in medium containing heterologous anti-rat R.E.I.S. diluted 1:20. 28X.

locytes form the most peripheral circle of such outgrowths. Macrophages and monocytes are found concentrated about half way beyond the middle of the zone of outgrowth after 48 hours of incubation while fibroblasts whose locomotor activity is the slowest of the cells under consideration are still intimately associated with the explant.

Photomicrographs of cultures in certain experimental conditions revealed a prominent ring of cells approximately midway between the explant and the periphery of the outgrowth. Such "coronas" have been represented in Figure 2. This response may be the result of an acceleration in the migration rate of macrophages and monocytes or of the activation of a larger number of such cells in the explant.

In certain situations strong concentration of R.E.I.S. produced marked clumping of migrating cells (figures 3,-4, 5, 6, and 9). This phenomenon was usually accompanied by restricted outgrowth. As a first approach in defining R.E.I.S. activity, the restriction of outgrowth and clumping of cells were found to be the most useful endpoints observed thus far in our tissue cultures.

In the course of exploratory studies experimental data were accumulated on embryonic and post-embryonic splenic fragments in media containing homologous and heterologous R.E.I.S.† Dilutions ranging from 1:4 to 1:5000 were used but it soon became evident that with the methods available for the evaluation of outgrowths, significant results could not be identified at dilutions higher than 1:400.

For these reasons this report is limited to results which present clear evidence for the inhibitory action of R.E.I.S. when tested at low dilutions.

Clumping of cells was observed in all cultures containing at least 1:20 dilution of R.E.I.S. Due to the scatter of the most peripheral cellular migrants and to occasional disturbance of elements as a result of slight liquefication of the clots,

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†Homologous R.E.I.S.—Specific for tissue used as explant and for the animal species.

Heterologous R.E.I.S.—Specific for tissue used as explant but of a different animal species.

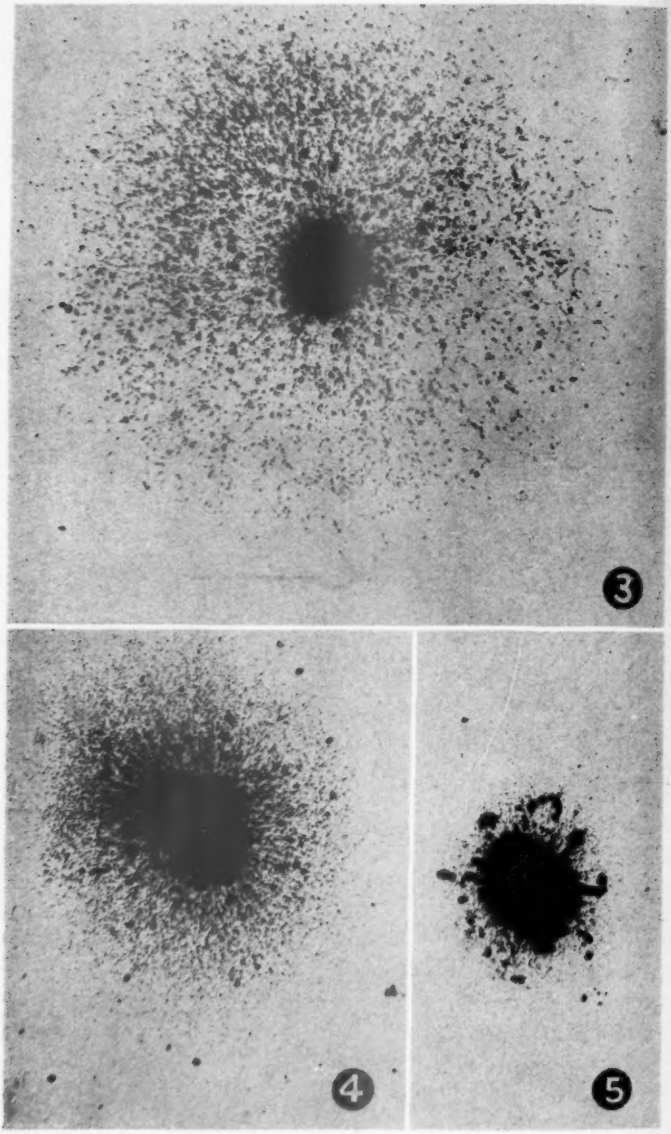

Fig. 3. Chick embryo—21 days. Spleen fragment in homologous anti-chick R.E.I.S. diluted 1:20. Note restriction of outgrowth and cell clumping. 28X.

Fig. 4. Chick embryo—12 days. Spleen fragment cultivated in medium containing anti-chick R.E.I.S. diluted 1:12. 28X.

Fig. 5. Chick embryo—15 days. Spleen fragment cultivated in medium containing anti-chick R.E.I.S. diluted 1:6. 28X.

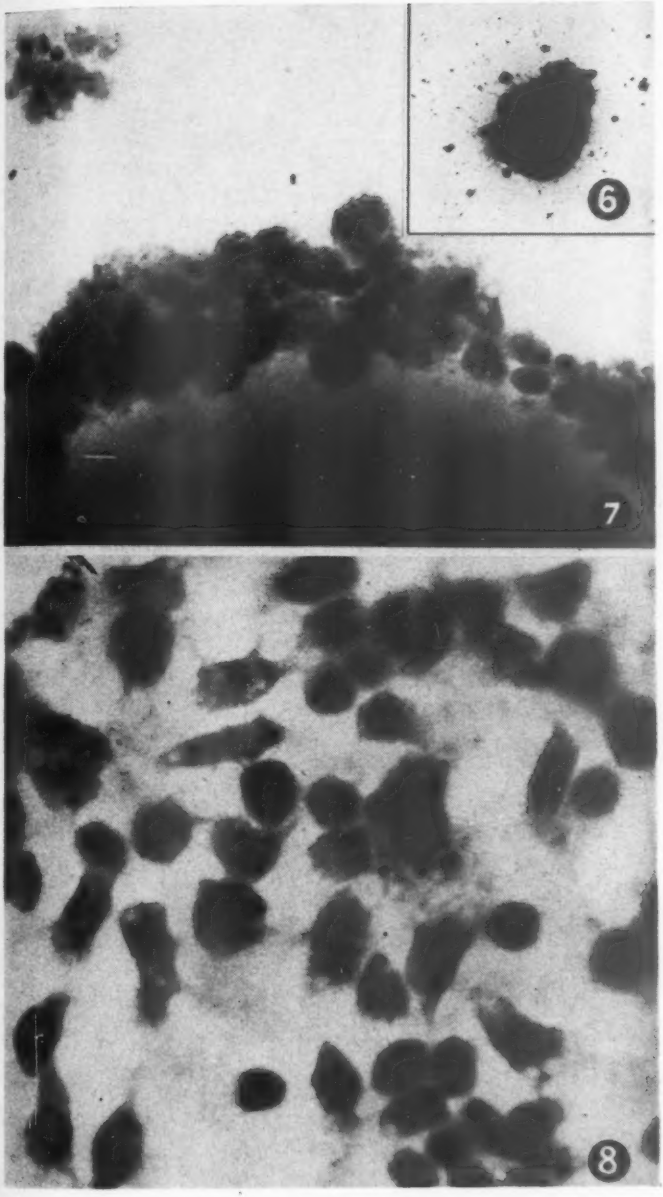

Fig. 6. Chick embryo—8 days. Spleen fragment cultivated in medium containing anti-chick R.E.I.S. diluted 1:4. 28X.

Fig. 7. Clump cells on the edge of the explant shown in figure 6. Cells filled with Chlorozol black. 650X.

Fig. 8. Control culture for explant in figure 7 showing Chlorozol black accumulation in uninjured cells. 650X.

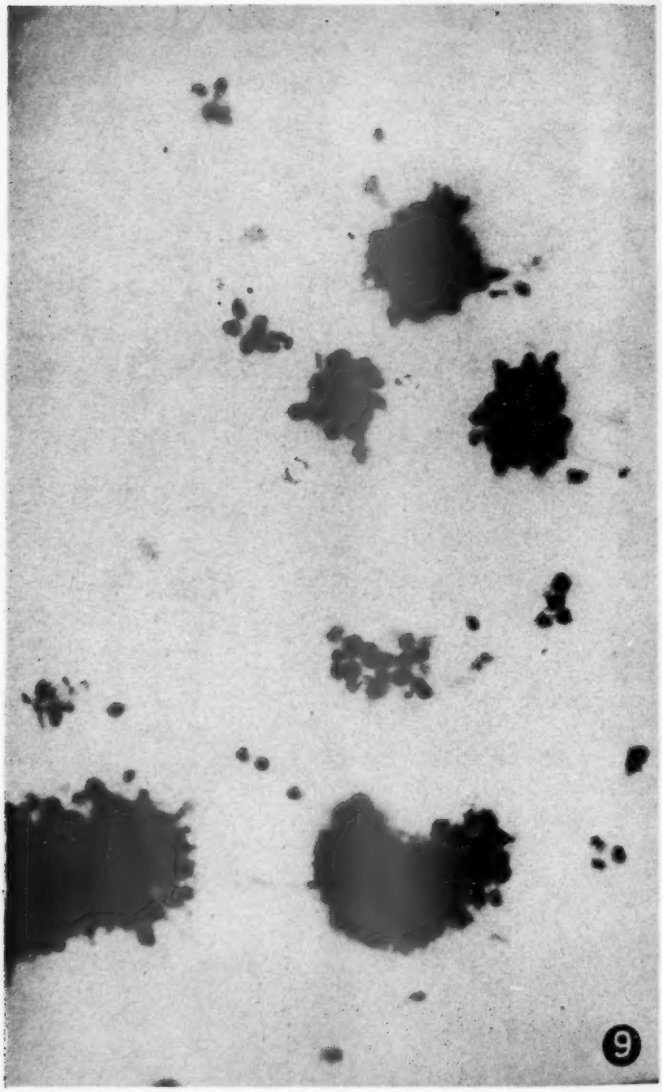

Fig. 9. Clumping of splenic cells under the influence of homologous R.E.I.S. 320X.

it was not found possible to make accurate measurements of the exact degree of inhibition of cellular migration. The amount of outgrowth is therefore simply graded by plus signs ranging from ± to ++++. The following table summarizes results obtained with chick spleens, *all of which showed clumping phenomena* when cultured at various concentrations of the immune serum.

R.E.I.S. Concentration	Experiment number	Chick age in days	Amount of outgrowth
1:4	XIV	8	±
1:4	XV	12	++
1:4	X	22	+++
1:4	XV	22	+++
1:6	IV	15	+
1:8	XIII	13	++
1:12	IV	10	+
1:12	V	18	+++
1:20	VIII	22	++
1:20	VIII	12	+++
Controls			++++

Limitation of outgrowth associated with cellular clumping is seen to be most marked at the higher concentration of R.E.I.S. and is also a function of the age of the splenic fragment. Thus 8-day spleens grown in R.E.I.S. at a concentration of 1:4 showed almost complete inhibition with marked clumping as compared with controls. Similar inhibitory action was observed for older spleens in association with R.E.I.S. at 1:6 and 1:8 but outgrowth was still found to be relatively vigorous at 1:4 for cultures of spleens from newborn animals. Detailed hematological studies may reveal significant differences under all varieties of experimental conditions.

Chlorozol black was actively accumulated by living microphages and macrophages (figure 8). R.E.I.S. in a concentration of 1:4 was not found to inhibit this process even in markedly clumped cells (figure 7). Granulocytes, identified as cells containing eosinophilic granules with Giemsa stain, were not found in cultures containing R.E.I.S. at concentrations of more than 1:240 in the series of experiments using 8-day spleens. In contrast, control cultures cultivated *in vitro* for three days contained a few cells (Lanchakoff, 1916). It seems, therefore, that

R.E.I.S. may have merely restricted the migration of granulocytes or may have prevented their differentiation.

Experiments with mammalian tissues were done with rat spleens from newborn animals or embryos at a late stage of development. Results with adult guinea pig spleens were reported in a preliminary note (Pomerat and Anigstein, 1944), but since the outgrowth of cells, even in controls, was not very dense, subsequent work was carried out on embryonic or newborn material.

Experiments on mammalian material which gave evidence of inhibitory effects are summarized as follows:

Species	R.E.I.S. Concentration	Experiment number	Age of the Animal
Rat	1:8	XI	20 day
Rat	1:20	XI	20 day
Rat	1:8	XI	Newborn
Rat	1:20	XI	Newborn
Rat	1:8	XII	Newborn
G. P.	1:10	I	Adult
G. P.	1:10	II	Adult

As in the case of chick experiments, all cultures carried out at a high concentration of R.E.I.S. showed marked limitation of outgrowth and clumping of cells. Rat spleens with R.E.I.S. at 1:20 showed somewhat less restriction of migration than corresponding cultures at 1:4.

In addition to results with homologous sera, heterologous sera at strong concentrations were observed to produce marked "coronas" in chick spleen cultures at a zone intermediate between the explant and the most peripheral migrating granulocytes (figure 2). This phenomenon probably represents stimulation approximately at the point of injury since similar "coronas" were observed in preparations containing relatively low dilution of homologous serum and showing some clumping of cells.

It is possible therefore that cultures could be "graded" according to the following scheme:

Grade	Outgrowth	Clumping	Corona	Exemplified by R.E.I.S.
1	±	++++	—	homologous 1:4
2	+	++++	—	homologous 1:8
3	++	+++	—	homologous 1:20
4	+++	++	++++	homologous 1:80
5	+++	+	++++	homologous 1:120
6	++++	—	+++	heterologous 1:4
			+++	homologous 1:240
7	++++	—	—	heterologous 1:8
			—	weak R.E.I.S. and control sera

It should be noted that this suggested scheme is based on impressions gained from the study of spleens from *young chicks* cultivated in serial dilution of R.E.I.S. Considerations must necessarily be given to the complement-fixation titre of the sera. It was not found advisable to "grade" the results obtained according to this plan since it would necessarily have remained a somewhat "impressionistic" process and because it is believed that a more quantitative method in the course of development will soon be available.

In analyzing the results of R.E.I.S. action on spleen fragments, other factors which might be involved were considered.

Since the concentration of embryonic extract is known to exert a marked influence on true growth in tissue cultures (Willmer, 1935), it became important to determine whether this might influence the reactions.

Embryonic extract at a final concentration of 25 per cent, 12½ per cent, 6¼ per cent, and 0 was employed in relation to three series of cultures:

(1) With anti-chick R.E.I.S. at 1:20.
(2) With normal rabbit serum at 1:20.
(3) A medium containing no serum.

Outgrowths from splenic fragments derived from embryos incubated for 12 days as well as from newly-hatched chicks showed no effects which could be attributed to the concentration of embryonic extract in the medium.

Hemoglobin is known to have an inhibitory effect on cells grown *in vitro*. The amount of blood in splenic fragments increases with the age of the organ, and since inhibition phenomena were not as marked in post-embryonic material it became important to test this factor. In several sets of experiments splenic fragments were carefully washed in five changes of Tyrode's solution over a period of 30 minutes before implantation in the plasma clot. This was not found to influence the results.

Discussion

In commenting on the possibility of modifying the activities of the reticulo-endothelial system, Cowdry states, "Our goal is to make the R. E. System not less efficient by blocking but more capable of acting as a defense mechanism. Repeated small stimulating doses of non-toxic substances might theoretically be helpful by mobilizing many phagocytic cells in readiness for some attack if we knew just when the attack was to occur." He concludes that the outlook to date (1938) "is not particularly promising."

Bloom (1927) has reviewed the earlier work dealing with the use of tissue cultures in observing cellular response to antigens in relation to sensitization and immunity. He states that various tissues have not been found to grow or, at best, grow slightly in cultures made with plasma from animals which had been immunized against these particular tissues. In his own experience, Bloom found that mesenchymal cells of embryonic rabbit lung, partially differentiated in the histiocytic direction, could be mobilized by an antigen such as pigeon erythrocytes.

In a more recent review of the literature dealing with *in vitro studies* on immunity Parker (1938), has discussed contradictory results reported by various workers. He concludes that ". . . the production of antibodies outside of the body may be dependent upon certain reactions within the organism and that these reactions must reach a certain stage before the process can readily be completed *in vitro*."

While deserving especial elaboration beyond the scope of the present study, a few remarks involving malignant tissue seem pertinent to this discussion. As early as 1914, Lambert found that small doses of rat sarcoma induced an antibody reaction in guinea pigs of sufficient concentration to produce toxic effects on sarcoma cells cultivated in the plasma of such animals.

Of interest, also, are the results reported by Braunstein (1933) on the effect of *oral* administrations of "splendothelan," a mixture of spleen, bone marrow and thymus, as *antigens* in the development of immunity to malignant tumors. Serum of rats immunized with "splendothelan" are reported to have shown definite inhibition of tumor growth in tissue cultures.

In a recent study, Burke, Sullivan, Petersen and Weed (1944) have described ontogenetic changes in antigenic specificity of chick organs. Using a procedure not markedly different from that described by Marchuk (1943) these workers prepared antigens and immune sera of various organs which were tested by quantitative precipitin and complement-fixation tests as well as by the injection of antisera on the chorioallantoic membrane of developing chicks. Organs are reported to change antigenetically during ontogeny. For example, the 312-hour chick brain was found to contain an antigen common to adult brain, a second common to the 160-hour brain but not to the adult brain, and a third antigen common to several other organs. In the light of these important findings, the greater sensitivity of very young spleen to R.E.I.S. reported in our experiments may find explanation. The possibility that immune sera influence extremely young undifferentiated primitive splenic elements deserves further investigation.

Attempts to evolve therapeutically valuable spleen and bone marrow preparations have developed along two major paths: (1) by the direct use of extracts of these organs, or (2) by using an immune serum derived from an animal treated with organ suspensions. While the first procedure is of *antigenic* value, the second is based on *antibody* phenom-

ena and is exemplified by the work of Bogomolets and associates, and as investigated by us as R.E.I.S.

To the *antigenic* series belong Braunstein's "splendothelan" and a group of studies developed by Leake and others. In 1923 Leake confirmed Danilewsky, Krumbhaar and Musser, and Downs and Eddy, in reporting an initial drop in the erythrocyte count in rabbits injected intravenously with fresh spleen extract. This is followed by a prolonged rise. Bone marrow injections produce a direct rise of 15 per cent above the normal erythrocyte count. An elevation of nearly 20 per cent in erythrocyte count results from combined bone marrow and spleen extracts. Considerable increases in the number of circulating leucocytes, especially in the polymorphonuclear neutrophiles, follow administration of combined splenic and bone marrow extracts.

A splenic hormone was proposed by Leake and Bacon (1924) to regulate the activity of the adult erythropoietic system. The possibility of using dessicated red bone marrow and spleen therapeutically was explored with some success in certain types of anemia (Leake and Evans).

While the action of the direct use of antigens on the recipient animal suggests a comparatively simple mechanism from the immunological standpoint, the interpretation of results obtained with the use of immune sera (R.E.I.S.) presents a far more complex problem.

Since the antibodies contained in immune sera are specifically directed against the wide variety of cell types contained in spleen and bone marrow, it is expected that when used at strong concentrations they may exert deleterious effects on the structure and function of corresponding test tissues. Direct evidence of this phenomenon has been brought forward in this study.

In addition, evidence with tissue culture technique has already been accumulated by us demonstrating the stimulating effect of low concentrations of R.E.I.S. The work on this phase of the problem will be reported at a later date.

Summary and Conclusions

The work of Soviet workers in the development of antireticular cytotoxic serum (ACS) has been used as a basis for the present study. The term reticulo-endothelial immune serum (R.E.I.S.) is suggested.

Tissue cultures of chick, rat and guinea pig spleen from embryos and post-embryonic animals have been used in testing the action of homologous immune sera. These sera were obtained from rabbits immunized against spleen and bone marrow. At concentrations of at least 1:20 conspicuous inhibitory effects were observed. Such explants showed marked inhibition of outgrowth and clumping of migrating cells. The mechanism of these results perhaps can be interpreted as immunological phenomena.

Evidence is being accumulated by tissue culture methods as to the stimulating effect of low concentrations of R.E.I.S.

Emphasis is placed on the difference between using spleen and bone marrow directly as antigens and the use of immune serum prepared from them for therapeutic purposes.

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CYTOLOGICAL CHANGES IN THE ANTERIOR LOBE OF THE HYPOPHYSIS FOLLOWING MASSIVE DOSES OF ALLOXAN

THURLO B. THOMAS AND G. A. EMERSON*

The diabetogenic action of alloxan, first described by Dunn, Sheehan and McLetchie, has recently received much attention. The concept that this effect is due to degeneration of many β-cells in the islets of Langerhans has abundant support (Bailey and Bailey, Duff and Starr; Goldner and Gomori; Hard and Carr; Hughes et al.; Kennedy and Lukens; Ridout et al.). The initial hypoglycemic action of alloxan (Jacobs) may be due to liberation of performed insulin from these cells after damage by the agent (Hughes et al.; Ridout et al.). The rapid appearance of degenerative changes in β-cells after alloxan treatment has been stressed by the latter workers. This rapidity of action supports the generally accepted hypothesis that alloxan acts directly on the islet tissue. In addition to this effect, however, alloxan causes structural changes in the kidney (Dunn and McLetchie; Duff and (Starr), the liver (Goldner and Gomori; Lackey et al.) and the adrenal medulla (Hard and Carr; Duff and Starr). No changes following administration of alloxan have been recognized in the pituitary (Bunde; Dunn and McLetchie; Bailey, Bailey and Hagan; Duff and Starr) or the adrenal cortex.

The contributions of Houssay et al., Long and Lukens, Lukens and Dohan, Richardson and Young, Richardson and others suggested to us that the known interrelation of the anterior lobe of the hypophysis, the adrenal cortex and the pancreas should be studied further by cytological methods in alloxan diabetes. That endocrine glands other than the pancreas are involved in alloxan diabetes is a possible explanation of the finding (Orias) that subtotal (95 per cent) pancreatectomy does not increase sensitivity to alloxan diabetes.

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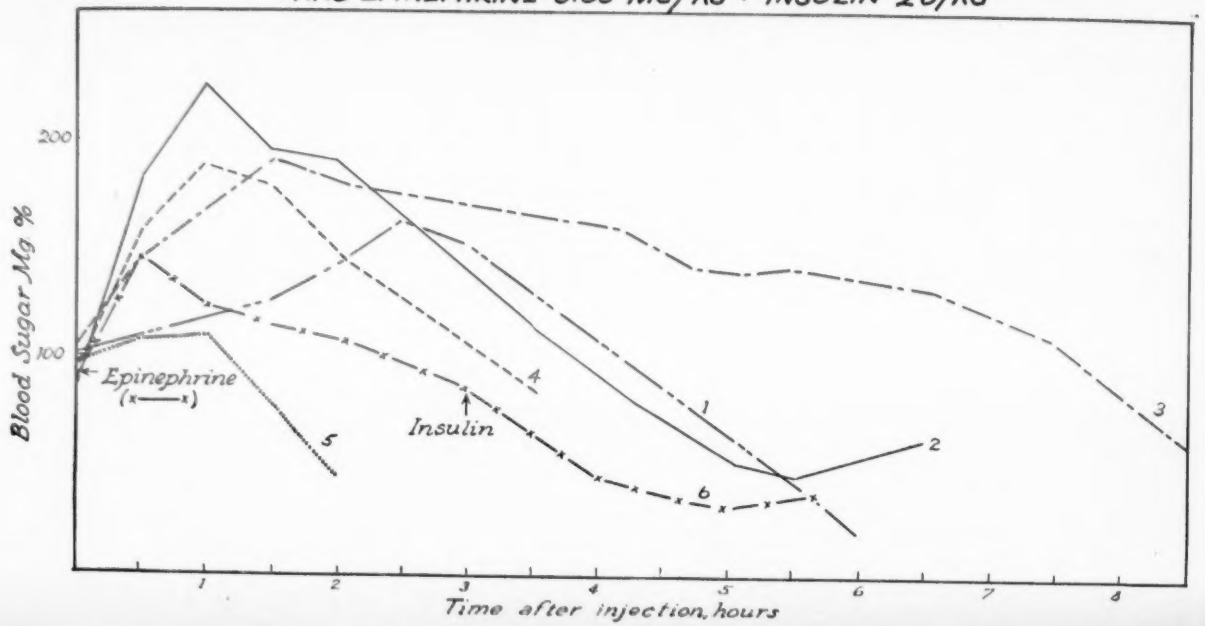
Observations on Rabbits

Acute Experiments. Six fasted adult rabbits were treated intravenously with 300 mg/kg. alloxan monohydrate. The content of reducing substances in the blood was estimated by the method of Hagedorn and Jensen before and at intervals after the injection of alloxan. These values are presented graphically in figure 1. All of the rabbits were killed shortly after the onset of hypoglycemic convulsions, at intervals noted by the termination of the curves in figure 1. The entire hypophysis was fixed in either Dawson's or Helly's fluid and the adrenals, the thyroid, the gonads, the tail of the pancreas and portions of the kidneys were quickly removed and fixed immediately in Helly's fluid.

Following dehydration in a graded series of alcohols the tissues were cleared in cedarwood oil and embedded in one of Waterman's hardened paraffins having a low melting point. Other than substituting 53°–55° C. "Bioloid" for "parowax," the mixture used was Waterman's SSB1 formula ("triple-pressed" grade stearic acid—16 per cent; spermaceti wax—3 per cent; bayberry wax—1 per cent; 53°–55° C. "Bioloid"—80 per cent). This mixture, which remained melted in an oven set at 50° C., is surprisingly hard and an excellent medium for cutting thin serial sections. These must be cut more slowly than with the ordinary paraffins, but the ribbons are smooth and the compression very slight. The hypophysis was cut serially at 3 μ and stained with Heidenhain's azocarmine as described in an earlier paper (Thomas). Cytological findings on organs other than the pituitary have been reported elsewhere.

As a control observation for the effects of alloxan on carbohydrate metabolism, a rabbit was injected intravenously with 0.05 mg/kg. of epinephrine hydrochloride, followed in 3 hours by a subcutaneous injection of 2 U/kg. of crystalline insulin. Effects upon blood sugar are given in figure 1. After hypoglycemic convulsions occurred, the rabbit was killed and tissues were removed and treated as with the rabbit's receiving alloxan. A photomicrograph of an azocarmine-stained

FIG. 1. COMPARISON OF ACUTE EFFECTS FOLLOWING ALLOXAN 300 MG/KG AND EPINEPHRINE 0.05 MG/KG + INSULIN 2 U/KG



section of the anterior lobe of the hypophysis of this rabbit is illustrated in figure 2. No significant abnormalities were found.

Figure 3 illustrates cytological changes found constantly in the anterior lobe of the hypophysis of all rabbits treated with alloxan. Marked degenerative changes in the basophilic cells were noted, although not all basophils were equally affected. Severe pyknotic changes were seen in many of the nuclei of basophils, with clear areas of degranulation surrounding the nucleus and at the periphery of the cells. Occasionally complete destruction of individual basophils was seen. In contrast, there was little or no severe damage to the acidophils or to the basophils of the pars intermedia.

Prolonged Experiments. Ten fasted adult rabbits were given 200 mg/kg. of alloxan monohydrate intravenously. Blood sugar was estimated before injection and at frequent intervals after injection for periods up to 29 days. One rabbit died within 12 hours after injection and was not included in the cytological study. The remaining 9 all developed persistent hyperglycemia and six were killed at intervals of 44, 68, 118, 144, 192 and 289 hours after the injection. Blood sugar levels at the time the rabbits were killed were 444, 710, 648, 460, 500 and 328 mg. per 100 ml., respectively. Tissues of these rabbits were treated as in the acute experiments.

Changes in the basophilic cells of the anterior lobe of the hypophysis of these rabbits were of less severity than in the acute experiments with a larger dose of alloxan. Nevertheless, many basophils showed the same striking degenerative changes. In Bunde's experience, there is no significant pathology of the pituitary in rats treated with 200 mg/kg. of alloxan and killed at shorter intervals after injection than were used in the present study.

Observations on Rats

Rats were treated subcutaneously with 400 mg/kg. alloxan monohydrate and killed at intervals of 1 to 5 hours after injection. The adrenals, pituitary and pancreas were re-

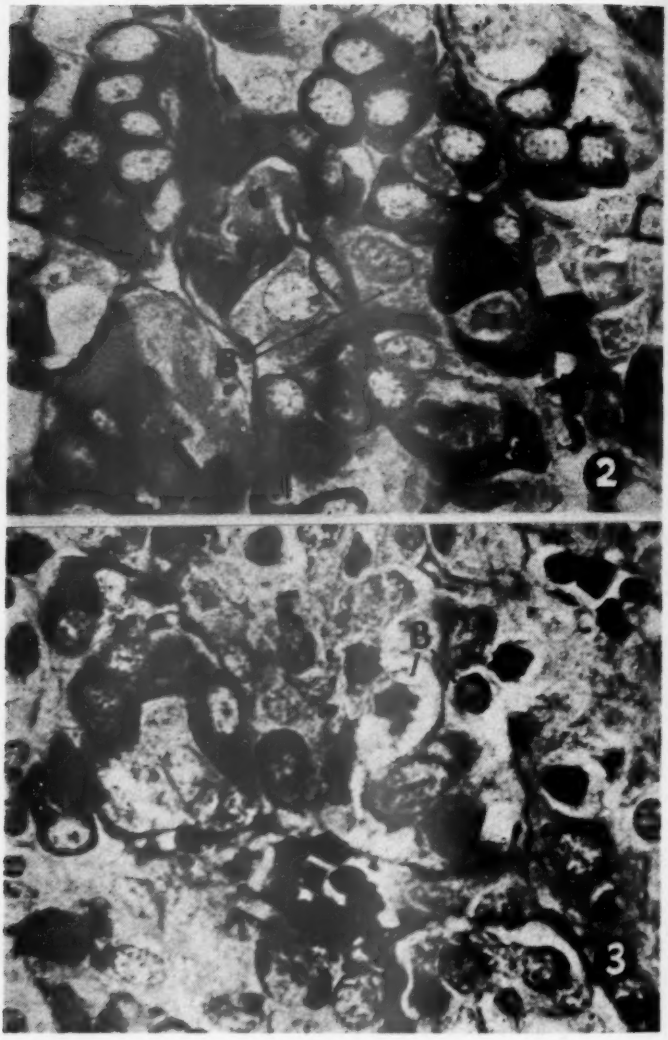


Plate I

Explanation of Figures

Fig. 2. Anterior lobe of pituitary from rabbit No. 6 treated with epinephrin and insulin (see Fig. 1). Note normal appearance of B cells. 1100X.

Fig. 3. Pituitary of rabbit given 300 mg/kg. alloxan intravenously. Note marked degranulation and pyknotic nuclei of B cells. 1100X.

moved and treated immediately in the same way as the rabbit tissues.

Cytological changes similar to those found in the anterior lobe of the rabbit hypophysis were found consistently in the basophils of the rat pituitary. The normal picture in an untreated rat and the abnormalities found after alloxan treatment are illustrated in figures 4 and 5.

Preliminary cytological study of the effects of alloxan has been made with rats under altered physiologic states. Rats were treated with 400 mg/kg. alloxan monohydrate after bilateral adrenalectomy 9 days previously, and also after complete pancreatectomy 12 hours previously. Other rats were given intraperitoneal injections of fresh adrenal cortical material, adrenal cortex extract (Upjohn) or massive doses of glucose. Findings in these animals demonstrate that the changes observed in the anterior lobe of the pituitary after alloxan treatment are not due to hyperglycemia; that large amounts of adrenal cortical extract (Upjohn) or fresh adrenal material do not produce the same changes; and that the effects of alloxan on basophils of the anterior lobe are not prevented by ablation of the adrenals of the pancreas.

In some of the rats, and in some rabbits treated with 300 mg/kg. alloxan, large patchy areas of complete necrosis were found in the adrenal cortex. A typical example is shown in figure 6.

Discussion

The present study describes cytological changes in the basophilic cells of the anterior lobe of the hypophysis, and in the cells of the adrenal cortex. Despite the relation of these two glands to diabetes, there is still no valid evidence that they play a direct role in the mechanism of alloxan diabetes; the changes found here may be explained as indications of a general cytotoxic action of alloxan. Contrariwise, there is not yet satisfactory proof that the diabetogenic action of alloxan is through a direct effect upon the β-cells of the islets; the speed of action of alloxan cannot be taken as proof, since extremely rapid changes in tinctorial properties

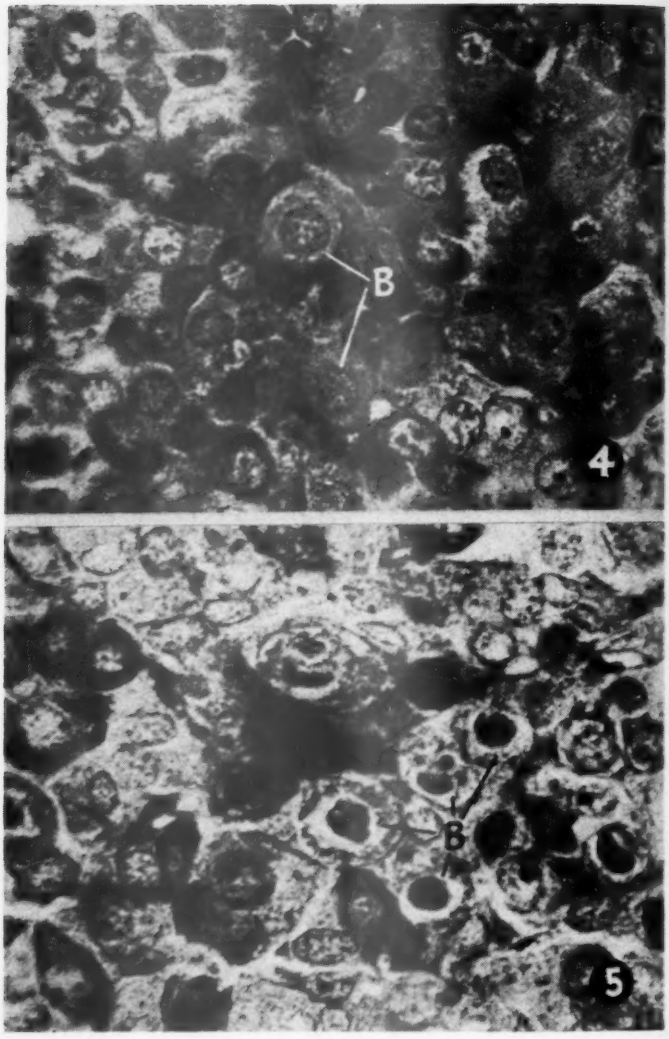


Plate II

Explanation of Figures

Fig. 4. Pituitary of control rat. Note normal granulation and nuclei of B cells. 1100X.

Fig. 5. Pituitary of rat given 400 mg/kg. alloxan subcutaneously and killed three hours later. Note characteristic damage to nucleus and cytoplasm of the B cells. 1100X.

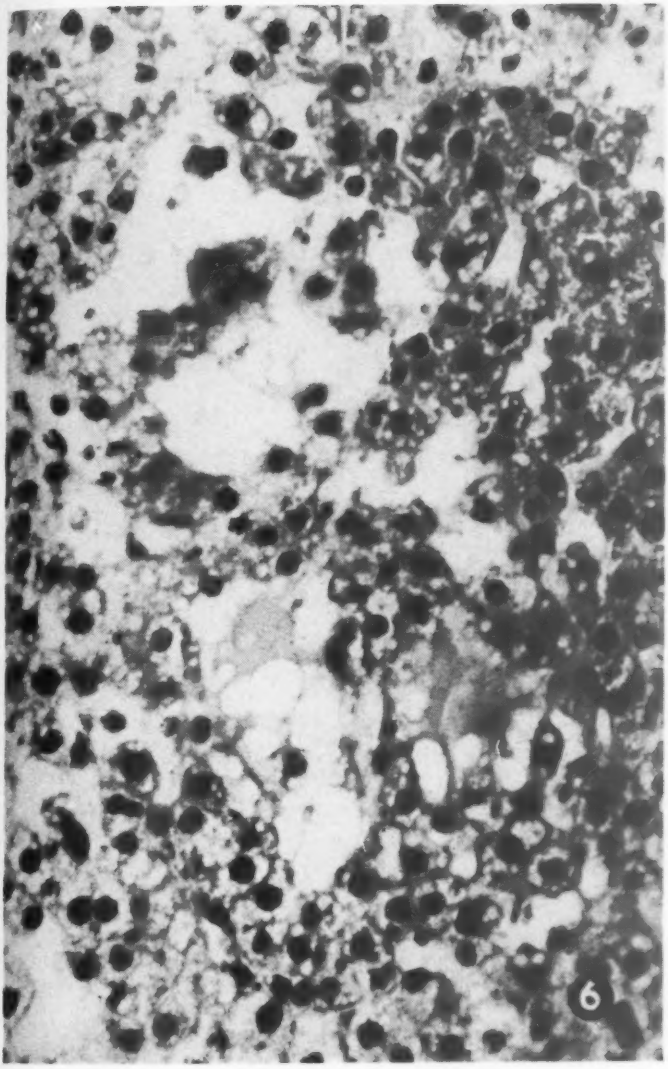


Plate III

Explanation of Figure

Fig. 6. Portion of fasiculata of the adrenal cortex of a rat treated with 400 mg/kg. alloxan subcutaneously. Note necrotic areas. 600X.

and function of the cells of the anterior lobe are known to occur in other instances; e.g., ovulation in the rabbit (Friedgood and Dawson).

Against the view that changes in the basophils of the anterior lobe are an expression of general cytotoxic action of alloxan is the perfect integrity of the basophils of the pars intermedia which presumably have been exposed to the same insult. Further, the rapidity of the diabetogenic action of alloxan does not necessarily favor either a direct or an indirect mode of action; in either case, the agent is so unstable in the body that its effects must be produced quickly or not at all, and these effects could be either on the pancreas or on other tissues which can act powerfully on the pancreas, to persist for a long time after the alloxan itself has been destroyed.

Summary

Cytological study of the anterior lobe of the hypophysis and the adrenals of rabbits and rats given massive doses of alloxan demonstrates severe degenerative changes in the basophils of the pituitary and in some instances areas of necrosis in the adrenal cortex. Implications of these findings for the mechanism of alloxan diabetes are discussed. The present findings are inadequate to substantiate a theory of indirect action, but there is also no satisfactory proof that alloxan is diabetogenic through a direct action on the β-cells of the islets of Langerhans.

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CALLING ATTENTION TO:

Items of Possible Interest to Friends of Our Effort

Happy Holidays, 1944

1. Philosophers Look to Scientisti: Columbia University Press sponsors significant group of philosophical efforts attempting to apply implications of current knowledge to our faiths and beliefs: Ray Lepley's Verifiability of Value (N.Y., 1944, suggesting scientific basis for ethics); L. S. Eby's *The Quest for Moral Law* (ditto), and symposium edited by Y. H. Krikorian, *Naturalism and the Human Spirit* (ditto). But, alas, none refer to C. H. Waddington's *Science and Ethics: An Essay and a Disscussion* (London, Unwin, 1942). The difficulties of western science in the philosophical development of the future are implied in the essays edited by C. A. Moore, *Philosophy East and West* (Princeton, 1944). The Princeton Press asks if you have discovered S. Kierkegaard (12 volumes available)? Seems to have been a rare Christian. A. E. Morgan offers fine appreciation of Edward Bellamy (1850–98), author of significant *Looking Backward* (Boston, 1888), issued by Columbia Press (N.Y., 1944).

2. Blood and Shock: E. Mylon, C. W. Cashman and M. C. Winternitz summarize succinate and plasma therapy of turniquet shock (*Am. J. Physiol.*, 142:299, 1944). I. H. Page describes cardio-vascular changes resulting from severe scalds (*ibid.*, p. 366). J. E. Davis reports 3 oral doses 10 mgm/Kg chlorine chloride daily reduces rbc's 30 per cent in normal dogs in 2–3 months and that liver or stomach orally corrects this hyperchromic anemia rapidly while atropine does so slowly (*ibid.*, p. 402). A. E. Shaw & Co. emphasize hematocrit and plasma protein estimations in controlling infusions for shock therapy (*Med. J. Austral.*, 2:301, September 16, 1944). Excellent symposium on blood: G. L. Taylor and R. R. Race and W. T. Morgan on blood groups and Rh.; C. J. Britton on anemias; H. Scarborough on hemoglobinometry; and E. P. Sharpey-Shafer on dynamics of hemorrhage (*Brit. Med. Bull.*, 2:160–192, 1944).

3. Choline Enzymes: J. F. Fulton and D. Nachmansohn & Co. suggest release of acetylcholine intracellularly depolarizes cell membrane rendering it permeable to all ions, thus generating action potential current to stimulate adjacent region and show parallelism between choline esterase activity and voltage of action potential (*Science*, 97:569, 1943); 100:454, November 17, 1944). F. Schutz shows high cholinergic activity is a factor in epilepsy (*Quart. J. Exp. Physiol.*, 33:35, 1944).

4. Bacteriostatics and Antibiotics: W. T. Salter gives full review of significance of antibiotics and bacteriostatics in blood and body fluids (*New Eng. J. Med.*, 231:651, November 9, 1944). A. Winkler confirms local anesthetic inhibition of sulfonamide action (*Zntralb. bakt. parasit. infekt.*, 151:106, 1944). D. G. Evans & Co. find p-methyl-sulfonyl-benzamidine HCl highly useful IM against Cl. welchii and other gas formers

(*Lancet*, 2:523, October 21, 1944). Skin sensitization to sulfonamides locally may be treated and prevented by low dosage orally, say B. C. Tate and L. Klorfajan (*ibid.*, p. 553, October 28, 1944). M. J. Romansky and G. E. Rittman's method of prolonging penicillin action by IM injection of peanut-oil and beeswax mixtures (*Science*, 100:196, September 1, 1944) is confirmed by G. W. Raiziss (*ibid.*, p. 412, November 3, 1944), in suggesting single injection from oral injection daily. A. H. Free & Co. offer evidence of effective absorption of penicillin from oral administration (*ibid.*, p. 431, November 10, 1944). M. Trumper & A. M. Hutter apply ice bag to area of penicillin injection to delay absorption and thus to prolong action (*ibid.*, p. 432). See special penicillin issue *Brit. J. Surg.* (32:109–224, July supplement, 1944) with fine color pictures in E. Cutler's article, and H. Florey's principles of treatment.

5. Looking to a Happier New Year: Our own C. M. Pomerat and L. Anigstein are first to offer experimental check on Russian anti-reticular cytotoxic sera (*Science*, 100:456, November 17, 1944). Our own G. A. Emerson notes limiting effects of privine (*J. Pharmacol.*, 82:42, 1944). R. M. Stecher continues keen studies on mechanism of inheritance of Heberden's Nodes (*J. Clin. Invest.*, 23:699, 1944). P. Cristol & Co. note relative rapidity of hydrolysis of monohalogenated acetic acids, confirming probability of negligible toxicity if used as food and beverage stabilizers (*Bull. Sci. Chim.*, 11:58, 1944; *Univ. Calif. Publ. Pharmacol.*, 1:397, 1941). Great group of sex hormone reports form appropriate memorial to Edgar Allen (*Yale J. Biol. Med.*, 17:1–349, October, 1944). Note second Ed. M. A. Pei's *Languages for War and Peace* (S. F. Vanni, 30 W. 12th, N.Y., 1944, $5.00). Congratulations to Nobelates Dam, Doisy, Erlanger and Gasser! More next year!

January, 1945

1. More Books: Reinhold issues M. Sahyun's *Outline of Amino Acids and Proteins* and P. J. Flagg's *Art of Resuscitation*. Farm Foundation, Chicago, offers *Medical Care and Health Services for Rural People*. Grune & Stratton, New York, publish L. Lichwitz's *Pathology and Therapy of Rheumatic Fever* (with full bibliography). C. C. Thomas (Springfield, Ill.) puts out third edition of R. Major's fine *Classic Descriptions of Disease*, L. Loeb's *Biological Basis of Individuality*, and P. McDonough's *Poet Physicians*. Nicholson of London offers B. Graves' *Comments on Medicine, Surgery and Education*. Faber also of London prints H. Renner's heavy tome on *The Origin of Food Habits*. Heineman still of London sells A. E. Wright's *Studies on Immunization, Second Series* (with Chemotherapy appendix). Livingstone of Edinburgh has W. Sargant and E. Slater's *Physical Methods of Treatment in Psychiatry* (!). Almquist of Uppsala offers biography of *The Swedberg* (1884–1944) with collection of biochemical papers. J. Vicente of Buenos Aires issues F. J. Manfredi's *Injertos Paratioideos*. El Ateneo, also of Buenos Aires, offers P. Negroni's *Micosis Cutaneos y Viscerales*. E. Braun-Menendez & Co.'s *Hipertension*

Arterial Nefrogena, A. J. Bandoni's *Terapeutica Antiparasitaria*, E. B. Del Castillo's *Endocrinolgia Clinica*, and Vols. 3 and 4 on symptoms and treatment of A. Gareiso's *Manual de Neurologia Infantil*. Yale Press issues Woglom's translation of C. Oberling's *Riddle of Cancer*. Hadham of New York sells F. Rider's *The Scholar and the Future of the Research Library*. Hopkins Press, Baltimore, issues H. Friedenwald's *Essays*.

2. More Antibiotics: S. Waksman & Co. isolate chaetonin from fungus Chaetonium cochliodes, which like penicillin is gram-positive effective and contains N and S (*J. Bact.*, 48:527, 531, 1944). Waksman's discussion on distribution of antagonistic fungi in nature is in *Mycologia* (35:47, 1943). G. F. Gause & M. Brazhnikova (Moscow) recommend 500 gamma per cc. solution locally of gramacidin S for infected wounds (*Nature*, 154:703, December 2, 1944). Merck's H. B. Woodruff finds streptothricin effective against mycobacteria *in vitro* (*Proc. Soc. Exp. Biol. Med.*, 57:88, 1944). Merck's H. J. Robinson indicates streptomycin's advantages over streptothricin (less toxic, more active) (*ibid.*, p. 226). S. Waksman then notes activity of streptomycin on M. tuberculosis (*ibid.*, p. 244). W. T. McClosky and M. Smith show sensitizing properties of penicillin (*ibid.*, p. 270). H. J. Greene and G. L. Hobby find penicillin passes placenta (*ibid.*, p. 282). E. B. McQuarrie & Co. report full study of penicillinase (*Arch. Biochem.*, 5:307, 1944).

3. More on Trauma: M. Prinzmetal & Co. suggest two types of burn shock, one due to local fluid loss (*Surg.*, 16:90, 1944), and recommend sulfamerazine locally and systemically immediately after crush injuries, to prevent shock (*ibid.*, p. 914). A. Forero & Co. correlate cardiac injury with extent of precordial trauma (*Rev. Argentina Card.*, 11:77, 1944). C. G. Tedeschi shows same for brain and head trauma (*Proc. Soc. Exp. Biol. Med.*, 57:264, 1944). E. G. Bywaters and J. K. Stead show renal failure when myohemoglobin, from muscle crush, is excreted in acid urine (*Quart. J. Exp. Physiol.*, 33:53, 1944).

4. Just More: S. J. Sarnoff and E. J. Poth find succinylsulfathiazole protective to bowel after blocking venous return (*Surg.*, 16:927, 1944). A. S. Wiener describes the RH series of allelic genes (*Science*, 100:595, December 29, 1944). E. Boehm and R. Williams recommend propyl gallate as effective anti-oxidant (*Quart. J. Pharm.*, 17:171, 1944). H. H. Anderson and T. K. Chuan find mapharsen effective amebacide *in vitro* (*Am. J. Trop. Med.*, 24:367, 1944). L. W. Kinsell & Co. recommend 12.5 mgm daily testosterone proprionate to prepare thyrotoxic patients for operation, in addition to iodine and thio uracil (*J. Clin. Invest.*, 23:880, 1944). G. N. Lewis and M. Kasha offer important concept on phosphorescence and triplet state (*J. Am. Chem. Soc.*, 66:2100, 1944). H. F. Blum gives evidence against sterol conversion hypothesis of carcinogenesis by ultraviolet radiation (*J. Nat. Cancer Inst.*, 5:89, 1944). J. B. Johnson and W. S. Wallace show how Tb in medical students may be controlled (*Texas S. J. Med.*, 40:428, 1944). N. F. Maclagan offers serum colloidal gold reaction in diagnosis of parenchumatous liver disease (*Brit. J. Exp. Path.*, 25:15, 1944; *BMJ*, 2:363, 1944), and also thymol serum turbidity

test (*Nature*, 154:670, November 25, 1944). B. D. Davis discusses variety of biologic false positive serologic tests for syphilis (*Med.*, 23:359, 1944). Young Turk abstracts appear (*J. Clin. Invest.*, 23:926, 1944). J. T. King & Co. confirm Taylor's work on tumor production by inoculation of tumor filtrate (*Proc. Soc. Exp. Biol. Med.*, 57:3, 1944). L. C. Miller and M. L. Tainter embellish ED_{50} idea (*ibid.*, p. 261). How about "certain safety factor"?

FEBRUARY, 1945

1. SYMPOSIA: Covering all phases of nutrition, and led by Sir Edward Mellanby (*Brit. Med. Bull.*, 2:202–254, 1944). Very important one sponsored by New York Academy of Science on energy relationships in enzyme reactions, by J. S. Fruton, E. G. Ball, M. Bergman, H. M. Kalckar, O. Meyerhof, and C. V. Smythe (*Ann. N. Y. Acad. Sci.*, 45:357–436, 1944). N. Y. Academy holding important one on hypertension, February 9 and 10, at American Museum of Natural History, N.Y. F. A. Lawes and B. Williams hold forth on fatigue (*Med. J. Austral.*, 2:501–507, November 11, 1944).

2. GAS: Look carefully into E. N. Harvey & Co.'s important studies on bubble formation in animals (*J. Cell. Comp. Physiol.*, 24:1, 23, 117, 133, 257, 273, 1944). J. W. Bean fully reviews effects of oxygen at increased pressures (*Physiol. Rev.*, 25:1, 1945).

3. CANCER: Add symposium by staff of Barnard Free Skin and Cancer Hospital (*Surg. Clin. North America*, Saunders, Philadelphia, 1944, pp. 981–1268). R. Leuchtenberger & Company report "folic acid" (L. casei factor) IV injections cause regression and prevention of spontaneous mouse breast cancers (*Science*, 101:46, January 12, 1945). V. R. Potter & Co. show that energy metabolism enzymes increase 3 fold after birth (*Cancer Res.*, 5:21, 1945). Note M. J. Shear & Co.'s series of articles on chemical treatment of tumors with hemorrhage producing polysaccharide from Serratic Marcescens (B. prodigiosus). These are summarized in clinical report (*J. Nat. Cancer Inst.*, 5:195, 1944), and refer to W. B. Coley's work (*Glasgow M. J.*, 126:49, 128, 1936) and G. Schwartzmann's *Phenomenon of Local Tissue Reactivity* (Hoeber, N.Y., 1937; *Cancer Res.*, 4:191, 1944). F. Duran-Reynolds shows importance of host resistance factors in enoplastic reactions to rabbit fibroma virus (*Cancer Res.*, 5:25, 1945). J. P. Greenstein and F. M. Leuthardt describe degradation of cystine peptides by exocystine desulfurase (*J. Nat. Cancer Inst.*, 5:209, 1944); *Science*, 101:19, January 5, 1945). F. E. Chidester writes on *Nutrition and Cancer* (Lancaster Press, Pa., 1944).

4. THERAPY: L. Loewe recommends combined use of penicillin and heparin in subacute bacterial endocarditis (*Canad. Med. Asso. J.*, 52:1, 1945). A. F. Coburn discusses control of streptococcus hemolyticus (*Mil. Surg.*, 96:17, 1945). L. W. Smith and A. E. Livingston recommended 1 per cent chlorophyll ointment containing 250 units penicillin per gram for promotion of wound healing (*Am. J. Surg.*, 57:30, 1945). D. Greiff & Co. find para-amino bensoic acid orally effective vs. murine typhus in

mice, and PBA and penicillin rickettsiostatic, while NaFl is the reverse! (*J. Exp. Med.*, 80:561, 1944). F. M. Allen summarizes treatment of surgical shock and embolism by preventing fluid loss, replacing fluid lost, and local and general refrigeration (*J. Internat. Coll. Surg.*, 7:423, 1944). Note H. McIlwain on theoretical aspects of bacterial chemotherapy (*Biol. Rev. Cambridge Philosoph. Soc.*, 19:135, 1944).

5. FURTHERMORE: J. W. Bartholomew and W. W. Umbreit report that ribonucleic acid with magnesium and cell protein is responsible for gram positive staining, and that sulfhydryl groups are involved (*J. Bact.*, 48: 567, 1944). C. Pinto recommends saponin for efficient and economical killing of snail carriers and cercaria of Schistosma in streams (*Mem. Inst., Oswaldo Cruz*, 40:210, 1944). B. D. Davis discusses variety of biologic false positive serologic tests for syphilis (*Med.*, 23:359, 1944). R. M. McKenna notes psychosomatic factors in skin disease (*Lancet*, 2:679, November 25, 1944). In swell discussion of structure and permeability of blood capillaries, J. F. Danielli and A. Stock conclude most substances reach tissue cells after filtration through pores in intracellular cement, and that edema follows Starling's hypothesis of protein gradient in pericapillary fluid and Poiseuille's equation that rate of flow is proportional to fourth power of capillary radius (*Biol. Rev. Cambridge Philosoph. Soc.*, 19:81, 1944). Note our E. H. Frieden's report on diffusion constant of penicillin suggesting molecular weight of 490 (*Science*, 101:21, January 5, 1945). Also F. T. Maher's *Reticulo-Endothelial System in Sulfonamide Activity* (Univ. Illinois Press, Urbana, 1944, $3.50. And S. Drake's discovery of A. B. Johnson and his works on language (*Rev. Gen. Semantics*, 1:239, 1944). While R. A. Woodbury and B. E. Abreu say yarns and sighs increase venous return, getting blood back to tired heart, ho-hum (*Am. J. Physiol.*, 142:721, 1944).